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ARS Insect Neurobiology Workshop Report and 5-year National Research Plan

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PREFACE

Some of the most serious problems of heavy reliance on the use of synthetic organic chemical insecticides have been (1) the development of resistance in many major insect pest species, (2) the phenomenon of pest resurgence, which occurs when an insecticide eliminates the natural enemies of the target insect and the pest builds up to greater numbers than previously existed, and (3) secondary pest outbreaks. Biological and biologically-based approaches hold great promise, along with integrated pest management and the judicious use of synthetic chemical insecticides, to help alleviate some of these problems.

In this context, insect neurobiology, and specifically the insect nervous system as a research focus, could provide the means for targeting the neuroendocrine system and lead to the control of a number of noxious insects by either altering their ability to fly, curtailing metamorphosis and development, interfering with certain specific physiological and metabolic processes, or disrupting sexual recognition and other related reproductive activities, or some combination of these strategies. An ARS Insect Neurobiology Workshop was convened in Beltsville, MD on October 27-28, 1992, to focus on the Agency's neurobiology research program as one of the avenues to help solve some of the applied research problems for insect pest control in efforts to decrease heavy reliance on chemical insecticide use.

At the workshop, a nationally-coordinated ARS insect neurobiology research plan was developed in order to facilitate a unified team effort, to clearly define program goals and objectives, identify each project's relevance and fit, and identify activities and time frames needed to reach objectives. The research action plan contained in this workshop report will help provide (1) program focus, (2) a basis for monitoring and evaluating program progress, (3) a basis for developing budget estimates and allocating resources, (4) responsiveness to the technology and problem-solving goals of the Agency, and (5) identification of technology transfer opportunities. Additionally, the research program plan will provide an important foundation for program strengthening and expansion, as resources can be made available, and for program coordination. The plan is a dynamic one and progress in reaching its goals will be reviewed periodically. Accordingly participants are expected to play a significant role in redefining essential activities, when necessary.

As a major theme of the workshop, it was emphasized that ARS is a mission-oriented research organization and is dedicated to solving high priority problems, using the most appropriate methods that yield solutions in a timely manner, at the lowest possible cost, while not sacrificing quality. Scientific knowledge in insect neurobiology will extend our view into the larger context of the entomological sciences; scientific discovery, no matter how basic, affords us an increasing perspective into this field, which we have only just begun to examine with a greater clarity.

Fundamental knowledge, of course, lies at our very scientific foundation; society often wants to realize immediate, tangible benefits on the investment. Thus, we must as scientists, continue to keep in mind as we pursue our particular objectives, what the practical spin-offs from the research might be. At the same time, one recognizes that all of the studies being undertaken in insect neurobiology by ARS scientists, and the scientific community, may not provide an immediate answer to insect control, but the fundamental knowledge being generated will most assuredly provide the necessary foundation for yielding applications that can take advantage of insect neurobiology, and thus provide additional weapons in mankind's insect control arsenal.

The National Program Staff expresses its gratitude and appreciation to all workshop participants and to all those individuals who contributed to the insect neurobiology research program plan. Special appreciation and thanks is accorded to the steering committee Chairperson and the steering committee members for their time and effort in helping to organize the working conference; Dr. Mark Holman, Chair, Dr. Mark Feldlaufer, Dr. Edward Dougherty, Dr. Terry Adams, and Dr. Peter Teal. The NPS is also very grateful to those individuals who served as session moderators, lead speakers, and team leaders. We are also indebted to Ms. Tara Peterson, Food Animal Protection Research Laboratory, College Station, TX for her valuable help in the research program plan coordination, as well as assembling the document report for publication.

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EXECUTIVE SUMMARY

A major goal of the Agricultural Research Service is the discovery, development, and application of unique, selective, and biorational insect control strategies. Many pest insect species have developed resistance to the synthetic pesticides currently in use, and the safety of those chemicals is increasingly being questioned by the public. However, these problems are not new. Over 30 years ago, scientists suggested seeking new methods of insect control based upon the unique differences between the neurobiology/endocrinology of insects and vertebrates. Recognizing the potential in this approach, ARS has supported research on insect neurobiology for almost 30 years and at the present time commits about $7 million annually to this effort.

An ARS-wide working conference on Insect Neurobiology Research was held in Beltsville, MD, on October 27-28, 1992. Based upon the information obtained from survey reports completed by ARS neurobiologists before the conference, the steering committee identified 11 research areas as the focus of the conference. Each research area was assigned to a team of scientists whose responsibilities included: (1) develop a research plan for that area; (2) present and defend that research plan at the conference; and (3) finalize the 5-year plan for that research area.

A series of research recommendations was generated by the conference participants. Research areas needing focus are: (1) synthesis of neuropeptide analogs and mimetics; (2) characterization of neuropeptide receptors; (3) molecular biology and genetics; (4) assays of biological activity; (5) regulation of degradation; (6) neurobiology and neurophysiology; (7) neuropeptides controlling water and ion balance; (8) immunocytochemistry; (9) mechanisms of neurochemical communication and their effects an diapause, development, and homeostasis; and (10) delivery systems for other than analogs and mimetics

Research gaps discussed at the conference included: (1) lack of orally active analogs, (2) lack of information on neuropeptides involved in cuticular tanning; and (3) lack of information on beetle neuropeptides and genetic information. Some of these gaps are presently being addressed by the synthetic analog scientists and the Manhattan, Kansas group who have contributed 5-year plans on cuticular tanning and beetle genetics.

The research action plan contained herein provides the framework for the next 5 years of insect neurobiology research in ARS. This plan is to be considered a dynamic one and will be modified periodically to reflect goals achieved or to adjust to technological advances unforeseen at this time.

OBJECTIVES AND CHARGE TO THE WORKSHOP

The overall charge to the Insect Neurobiology Workshop was to develop a nationally-coordinated ARS insect neurobiology research program plan that will help provide the necessary methodology, and team efforts to ultimately yield usable and acceptable control technologies for management of noxious insect pests, The specific objectives were to:

1. Delineate progress, research gaps, and scientific and technological objectives of insect neurobiology research.

2. Provide a forum for expressing views and ideas on both immediate and future research needs.

3. Identify areas of mutual interest and avenues of cooperation.

4. Identify possible mechanisms of translating the knowledge and technology to the field.

INSECT NEUROBIOLOGY IN ARS: AN NPS PERSPECTIVE

Ralph A. Bram[1]

Insect neurobiological research has been conducted for over 25 years at a number of laboratories within the Agricultural Research Service (ARS). Initially, major emphasis was given to research on the identification, characterization, and synthesis of semiochemicals and peptide neurohormones. A key characteristic of this research has always been its multidisciplinary nature. Teams consisting of entomologists, chemists, molecular biologists, physiologists, zoologists, and microbiologists have worked together in one of the truly long-term, high-risk areas of research.

Within the past 5 years, this research has resulted in significant advancements in our understanding of the functional unit of the nervous system. Some examples of this remarkable progress included: identification of ecdysteroids associated with molting, sexual attraction, reproduction, and oviposition; isolation, sequencing, and synthesis of over 70 biologically active neuropeptides; partial characterization of peptide receptors; and elucidation of peptide biochemistry. Now we have come to the point of asking the question, "Why is ARS committing in excess of $7. million per year in this area of science?" Answers to this question are many: to reduce losses of agricultural products to insects; to provide a foundation for the development of unique, biorational methods of insect control; to discover new scientific principles of insect control; to develop novel, highly efficacious and wholly selective methods for the control of selected pests; or to develop fundamental knowledge. These answers should come as no surprise to you. They were taken from the mission statements of your own respective Research Units.

But what is the National Program Staff (NPS) perspective of insect neurobiology research? Simply stated, it is an approach to solving agricultural problems. Perhaps the title of this workshop should be: "Insect Neurobiology: A Foundation of Economic Entomology." To extend the NPS perspective, this workshop must address several basic questions: what will be done with fundamental knowledge gained from research in insect neurobiology?; how can this information contribute to integrated management systems for arthropods of agricultural importance?; how will new technologies be implemented - are there even delivery systems on the horizon that can capitalize on neurobiological discoveries?; or, how can insect neurobiological research interact with new ARS initiatives of the U.S. National Genetic Resources Program? Furthermore, each scientist should have his or her own vision of how their personal research will contribute to the solution of an agricultural problem.

The NPS perspective also includes the management philosophy of project accountability. Your project, commonly known as a CRIS, is a "contract" with the agency. This "contract," approved by both NPS and your respective Area Director, identifies the major programmatic and administrative guidelines for your research: (1) the problem to be addressed; (2) the objectives of your research; (3) the approach that will be taken; (4) the number of scientist years devoted to the project; (5) the annual funding that ARS will commit to the project; and (6) the duration of the research effort. Although these guidelines are flexible, project changes or reductions cannot be made unilaterally by the Research Scientist or the Research Leader. Changes to a CRIS can, however, be made through consultation with your National Program Leader and subsequently approved by line and staff officials. Most often, changes to a CRIS before the established expiration date are indicated when the objectives have been accomplished or when a new, higher priority problem emerges requiring redirection of resources.

I can assure you that NPS looks forward to the results of this workshop on insect neurobiology. The pre-workshop materials, provided by the steering committee, are excellent. Now, the time has arrived when we can collectively chart new directions for research to solve agricultural problems through neurobiological intervention or manipulation. There is no doubt that we will all benefit from the conclusions and recommendations of this workshop.

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SENSORY NEUROBIOLOGY (CHEMICAL SENSES)

Current Status

Behavior of insects is determined by the sensory input they receive from various stimuli in their environment and their readiness to respond as governed by their internal physiological state. Both the sense organs, and specialized receptor neurons or nerve cells associated with them have evolved to detect chemical and physical signals from conspecifics and plant or animal hosts, that are important for individual sustenance and species propagation. Chemical signals play a major role in host selection, feeding, mating, and oviposition. Species-specific chemical messengers must be detected and processed for each species. An understanding of the basic mechanisms of chemoreception by which insects detect and respond to chemical signals is tantamount to biorational control of insect behavior for survey or control measures; e.g. mating disruption or trap-out strategies. Chemical messengers may also be used to impart species specificity to attracticide devices, thus facilitating use of other control technologies, e.g. pathogens or neuropeptides.

Behavioral responses of insects to chemicals have been the subject of numerous investigations over the last century. Pioneering studies by Von Frisch and others on sensory responses of honey bees and other insects revealed integration of information from various sensory modalities, and the importance of this integration in discrimination. Quantitative studies of insect chemoreceptors awaited not only the availability of oscilloscopes, high impedence preamplifiers, and techniques for fabrication of microelectrodes, but also collective synergism of personalities and expertise of individuals at Columbia and Tufts Universities, and the Massachusetts Institute of Technology. Behavioral experiments by Dethier at Johns Hopkins University suggested the location of chemosensory sensilla in flies. The first successful recordings from single neurons associated with these sensilla were made by Hodgson (a post-doctoral fellow at Tufts with Roeder and former student of Dethier), Lettvin (at M.I.T.) and Roeder (at Tufts), and published in *Science* in 1955. Schneider and Boeckh in 1962 were the first to publish results of electrophysiological recordings from individual olfactory receptor neurons in adult insects. Research in insect olfaction mushroomed with the identification of the first insect pheromone, bombykol, which served as a reliable stimulus for receptor neurons in the sexually-dimorphic trichoid sensilla of male silkworm moths, *Bombyx mori*. An insect olfactory research unit set-up in the early sixties at the Max Planck Institute for Behavioral Physiology in Seewiesen, Germany, continues research in insect olfaction and behavior today. Other research groups in Regensburg, Germany; Paris, France; Trondheim, Norway; Lund, Sweden, and Worcester, Massachusetts have considerable efforts in insect olfactory neurobiology and behavior. More recently in the United States, a group was established within the framework of the Arizona Research Laboratories, Division of Neurobiology at the University of Arizona in Tucson to utilize "insect neural preparations to reveal fundamental neurobiological processes common to many or all animal species".

Neurobiological studies of insect olfaction have a long history within ARS. The first electrophysiological studies of insect olfaction in ARS were reported in *Nature* in 1968 by M.S. Mayer who determined that the blood-feeding bug, *Triatoma infestans*, possessed single receptor neurons which responded to human breath. The Insect Attractants, Behavior, and Basic Biology Laboratory in Gainsville, Florida was established in 1970 to provide increased emphasis on the discovery and utilization of new insect attractants, and to enhance research on neural and behavioral mechanisms of insect responses to them. Concurrently, V.E. Adler at the Biologically Active Natural Products Laboratory in Beltsville, Maryland had begun recording electroantennograms (EAGs) to determine the olfactory responsiveness of certain insects to selected odorants.

Currently within ARS there are several individuals working more or less in the area of olfactory sensory neurobiology. Most recent studies have dealt with olfactory reception of pheromone components and host plant odors by economic insect species. J.C. Dickens has characterized olfactory receptor neurons for pheromone components and plant odors of several species of Coleoptera including the boll weevil, identified pheromone receptor neurons in the southwestern cornborer and the beet armyworm, and determined olfactory responsiveness of a parasitoid to host habitat odors. Dickens is currently investigating olfactory reception of potential pheromone components and plant odors by the tarnished plant bug, and doing comparative studies of olfactory receptor neurons for pheromones and host odors in the beet armyworm, the fall armyworm, and the southern armyworm. These studies have led to: (1) a better understanding of the boll weevil pheromone; (2) the discovery of green leaf volatiles from plants as synergists of the boll weevil pheromone and pheromones of other insects (in cooperation with Light, Jang, and others); (3) identification of the sex attractant pheromone of the southwestern cornborer; and 4) development of biologically-active analogs to modify receptor neuron responses and their associated behaviors. E.B. Jang has cooperated with D.M. Light and J.C. Dickens in studies of the antennal olfactory responsiveness of several economically important fruit flies to potential pheromone components and plant odors. Jang established the importance of accessory glands in modulating behavior of fruit flies, and studied the effects of benzodioxoles on *in vitro* biosynthesis and release of juvenile hormone by corpora allata in Mediterranean fruit flies. D.M. Light has determined the sensitivity and responsiveness of olfactory receptors of the alfalfa seed chalcid to host and nonhost plant volatiles. Light demonstrated that the attractiveness of fruit volatiles to Mediterranean fruit flies was correlated to fruit ripeness. The collaborative studies by Jang and Light have provided a better understanding of olfactory-mediated behavior of fruit flies and provide the bases for improved control or survey measures. M.S. Mayer has conducted basic research on pheromone reception by moths using the cabbage looper. Mayer characterized sex pheromone receptor neurons in the male cabbage looper and demonstrated their specificity at behaviorally relevant concentrations. Mayer and his coworkers were the first to demonstrate different morphological classes of trichoid sensilla and pheromone receptor neurons in male moths. Most recently, Mayer developed behavioral bioassays to demonstrate discrimination of pheromone blends by cabbage looper males. Mayer, Dickens, and others collaborated to determine the effects of certain pheromone analogs on receptor neurons of male moths. These studies have elucidated reception of pheromone components by male moths, have facilitated an understanding of discrimination of pheromone blends, and may be utilized in the application of pheromones in the field for survey or control measures, e.g. mating disruption.

Future Directions

At the present time there are several research thrusts in insect chemoreception, in general, and olfactory neurobiology, in particular.

1. Studies of receptor neurons for pheromones and plant odors . Research in this area is mainly aimed at elucidation of biologically meaningful chemical messengers from signaling conspecifics or potential hosts. In the past, a dichotomy existed between those who investigated pheromones and those investigating plant odors. Recent discoveries of effects of plant odors on pheromone receptor neurons, observed synergism between pheromones and plant odors, and the need for female attractants, has led to more integrated studies. ARS is well represented in this area of research. Our proposed five year action plan includes characterization of receptor neurons in five species of moths (JCD, DML and MSM), the tarnished plant bug (JCD), and potentially the Mediterranean fruit fly (EBJ, DML and JCD). Cooperative studies with chemists are planned to elucidate potential agonists or antagonists for identified receptor neurons (JCD, EBJ, DML and MSM). These studies are important not only for development of novel or alternative biorational control strategies for target species using behavioral chemicals and analogs, but also for identification of naturally-occurring chemical signals important in the behavior of pestiferous and beneficial insects.

2. Studies of binding sites and ion channels. Future studies are proposed in ARS to attempt to develop techniques for isolating binding sites from insect olfactory receptor membranes (JCD). Isolated binding sites and associated ion channels would facilitate studies of receptor neuron specificity and allow for a better understanding of contributions to neuronal specificity afforded by components of olfactory sensilla. These studies would provide further knowledge for design and evaluation of potential agonists and antagonists of insect olfactory receptor neurons. Arrangements for potential collaboration in these efforts have been made with R. J. O'Connell at the Worcester Foundation for Experimental Biology, Worcester, MA.

3. Studies of interneurons in the olfactory pathway and integration of sensory input. Multifiber and intracellular recordings from interneurons in fruit flies and the corn earworm are planned in an effort to determine discrimination of complex odors by these insects (DML). These studies would parallel current studies on processing of olfactory information in the olfactory pathway already in progress at the Arizona Research Laboratories, Division of Neurobiology, Tucson, the Institute for Zoology at University of Regensberg, Germany, and the Department of Ecology, Lund University, Sweden. Studies of information processing by the central nervous system of insects provide an understanding of neural mechanisms involved in discrimination of complex odors and integration of stimuli from various sensory modalities. From a practical aspect, if a particular transmitter were found in a specific olfactory pathway, agonists or antagonists of the transmitter might be used to disrupt communication. Another approach to brain function is being undertaken in which human psychological and psychophysical approaches will be applied (MSM). This approach takes into account knowledge of receptor function and behavior, and infers brain function.

4. Modulation of behavior by neuroactive substances . Investigations are planned on modulation of the switching from pheromone seeking to host seeking behavior in tephritid flies (EBJ). Research to date implicates the accessory gland as the source of chemicals involved in this switch. Modulation of behavior and sensitivity of chemoreceptors by blood-borne substances have been demonstrated in several instances. For example, an endogenous factor modulates sensitivity of olfactory receptors for a host attractant in female mosquitoes which is sufficient to explain initiation of host seeking behavior. Juvenile hormone may modify sensitivity of pheromone receptors in moths and beetles. Sensitivity of contact chemoreceptors may be modulated by hunger or nutritional deficiencies. Modulation of behavior and sensitivity of receptor neurons or neurons within the central nervous system is an area which may provide considerable new knowledge for a better understanding of insect behavior for biorational control measures.

5. Contact chemoreception. Research on contact chemoreceptors (taste receptors) involved in feeding will be investigated in tephritid fruit flies (EBJ). Studies of contact chemoreceptors are being pursued only in a few laboratories in the U.S., Canada, England and Japan. Characterization of contact chemoreceptor neurons in insects is important since the activity of these neurons may initiate or deter feeding. Several specific areas of research involving contact chemoreception currently under investigation include: (a) feeding deterrents; (b) the role of nutritional feedback in regulating feeding and receptor sensitivity; (c) transduction mechanisms; (d) processing of gustatory input in the suboesophageal ganglion; (e) neural coding; and (f) the importance of mixtures in host recognition. Research in each of these areas is necessary to better our understanding of host recognition, and acceptance or deterrence for use in integrated control of insect pests.

Sensory Neurobiology

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Determination of antennal olfactory responsiveness of target insects.	Begin studies of antennal olfactory responsiveness in *Spodoptera exigua*, *Microplitis croceipes*, and *Lygus lineolaris* . (JCD) Determine effects of new pheromone analogs in *Anthonomus grandis*. (JCD)	Continue studies begun earlier. (JCD) Begin comparative studies of *Spodoptera* species with *S. furgiperda* . (JCD) Test potential semiochemicals identified by cooperating chemists for antennal activity in *L. lineolaris* and other target species. (JCD)	Continue studies begun earlier. (JCD) Continue comparative studies of *Spodoptera* species with *S. eridania*. (JCD) Continue test of potential semiochemicals in *L. lineolaris* . (JCD)	Complete studies of antennal olfactory responsiveness of target insects. (JCD)	Test potential behavioral chemicals for antennal activity as needed for target pests. (JCD)
2. Identification of sensilla honsing receptor neurons for semiochemicals.	Determine sensillar types in *S. exigua*, and *L. lineolaris*, based on surface structure using scanning electron microscopy (SEM). (JCD)	Continue SEM studies begun earlier. (JCD) Begin comparative studies of *Spodoptera* species with *S. frugiperda* and *S.. eridania* . (JCD)	Continue SEM studies begun earlier. (JCD) Begin transmission electron microscopy (TEM) of antennal receptors in *L. lineolaris*, if necessary. (JCD)	Complete SEM studies on target pest species. (JCD) If necessary continue TEM studies on antennal receptors of *L. lineolaris*. (JCD)	Complete morphological studies of antennal sensilla in target pests. (JCD)

Sensory Neurobiology Cont.

3. Characterization of receptor neurons for semiochemicals in target insects.	Characterize receptor neurons for pheromone components in *Spodoptera* species. (JCD) Develop techniques for recording from single sensilla in *L. lineolaris* . (JCD)	Continue studies begun earlier. (JCD) Cooperate with chemists and other entomologists in identification of pheromone for *L. lineolaris.* (JCD)	Continue studies begun earlier. (JCD) If possible, develop techniques to record from receptor neurons responsive to plant volatiles in *Spodoptera* species and *L. lineolaris.* (JCD)	Continue studies begun earlier. (JCD)	Complete characterization of receptor neurons for *Spodoptera* species, and, if possible, *L. lineolaris.* (JCD)
4. Studies of receptor neuron characteristics through designed molecules for target neurons.	Consult with cooperating chemists as to chemical strategies and practical syntheses of potential agonists and antagonists for identified neurons. (JCD)	Synthesis of designed molecules by cooperating chemists. (JCD) Determine responsiveness of identified neurons in *A. grandis* to new pheromone analogs. (JCD)	Determine effects of designed molecules on antennal olfactory responsiveness, and activity of identified neurons. (JCD) Continue development of techniques for working with isolated receptor sites. (JCD)	Continue studies begun in year 3. (JCD) If possible test effects of semiochemicals and designed molecules on isolated receptor sites. (JCD) Continue dialog with chemists. (JCD)	Continue studies begun earlier. Continue dialog with chemists. (JCD)

Sensory Neurobiology Cont.

		Attempt to develop techniques for isolation of binding sites from olfactory membranes to facilitate analysis of receptor site specificity. (JCD) Continue dialog with chemists as additional neurophysiological information becomes available. (JCD)	Continue dialog with chemists. (JCD)		
5. Behavioral studies to determine activities of "key" compounds for target neurons and designed molecules.	Consult with cooperators regarding appropriate behavioral bioassays for semiochemicals. (JCD)	Conduct or develop bioassays for semiochemicals for *Spodoptera* species and *L. lineolaris* . (JCD) Determine behavioral effects of pheromone analogs and "key" plant volatiles on *A. grandis* behavior. (JCD)	Continue studies begun in year 2. (JCD) Complete studies with boll weevil pheromone analogs and key plant volatiles. (JCD)	Continue studies begun earlier. (JCD) Complete studies of behavioral responses of *A. grandis* to key plant volatiles. (JCD)	Continue studies begun earlier with *Spodoptera* species and *L. lineolaris.* (JCD)
6. Studies of behavioral switching by tephritid fruit flies (EBJ)	Determine if behavioral switch occurs both in irradiated and wild flies (EBJ)	Identify male accessory gland factors responsible for behavioral change (EBJ)	Determine role of ovaries in female behavior. (EBJ)	Continue studies begun earlier using other fruit flies and parasites. (EBJ)	Establish specific behaviors of fruit flies and their parasites that are modulated by neurohormones. (EBJ)

Sensory Neurobiology Cont.

7. Studies on pheromones of tephritid fruit flies, e.g. Mediterranean fruit fly.	Determine factors influencing behavior to pheromone, e.g. green leaf volatiles. (EBJ, DML)	Continue studies begun earlier. (EBJ) Begin studies of adult feeding behavior. (EBJ)	Continue studies begun earlier. (EBJ) Initiate studies of role of gustation in feeding. (EBJ)	Continue studies of feeding behavior. (EBJ)	Determine role of neuroendocrine factors in behavioral responses to pheromones and feeding. (EBJ)
8. Electrophysiological studies of chemoreception in tephritid fruit fliesand associated parasitoids (EBJ), and *Helicoverpa zea.* (DML)	Determine relationship between chemoreception and behavior in fruit flies and their parasitoids. (EBJ) Trap and identify volatiles from insects and host plants. (DML)	Determine receptor system receptivity to identified volatiles. (DML, EBJ) Begin structure/activity studies of tephritid receptors utilizing single cell recordings. (DML, EBJ)	Continue single cell recordings on other fruit flies. (EBJ, DML) Determine relationship between single cell responses and physiological changes. (EBJ)	Begin electrophysio-logical and behavioral studies of responses of parasitoids to host odors. (EBJ, DML) Laboratory behavioral bioassays of insect and host odors. (DML)	Continue studies begun earlier. (DML) Field behavioral bioassays of insect and host odors. (DML)
9. Physiological and biochemical studies of fruit fly growth and development.	Identify physical and chemical factors in diet that affect growth and development, e.g. pH, temperature, osmolarity. (EBJ)	Continue studies begun earlier. (EBJ) Determine titers of hormones during larval development. (EBJ) Begin nutritional studies. (EBJ)	Continue studies begun earlier. (EBJ) Optimize fruit fly diet. (EBJ) Initiate studies on ovarian development. (EBJ)	Continue studies begun earlier. (EBJ) Initiate studies on physiological control of fruit fly reproduction. (EBJ)	Study endocrine/ neuroendocrine control of development. (EBJ)

Sensory Neurobiology Cont.

10. Studies of discrimination of complex odors by central nervous system in fruit flies and *H. zea*.	Morphological studies of central nervous system. (DML)	Characterization of neurons by multifiber and intracellular recordings and staining. (DML)	Continue studies begun earlier. (DML)	Construction of neural network model. (DML)	Continue studies begun earlier. (DML)
11. Studies of neural modulators.	Isolate and identify receptors. (DML)	Continue studies begun earlier. (DML)	Determine mode of action in central nervous system. (DML)	Continue studies begun earlier. (DML)	Continue studies begun earlier. (DML)
12. Studies of pheromone discrimination by Lepidoptera, e.g. *Trichoplusia ni*, *Manduca sexta*, and *H. zea* .	Begin studies of single pheromone components. (MSM)	Continue studies begun earlier Begin studies of mixtures of two or more pheromone components. (MSM)	Begin studies based on results of neurophysiological studies. (MSM)	Continue studies of combined behavior and electrophysiology. (MSM)	Study effects of pheromones and neuroagonists. (MSM)
13. Characterization of responses of single antennal pheromone specialist receptor neurons in Lepidoptera, e.g. *T. ni*, *M. sexta* and *H. zea* .	Begin studies with single pheromone components. (MSM)	Continue studies begun earlier. (MSM) Begin studies with mixtures of pheromone components. (MSM)	Continue studies begun earlier. (MSM) Begin studies with congeners, isomers, and analogs. (MSM)	Begin studies of receptor function. (MSM)	Continue studies begun earlier. (MSM) Begin biophysical and biochemical studies of receptor function. (MSM)

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ECDYSTEROIDS

Introduction and Current Status

"Ecdysteroids" is a generic term for a large group of polyhydroxylated, Δ^7,6-ketosteroids that are found in plants, arthropods and other invertebrates. In insects, these compounds function as hormones, governing - as their name implies - ecdysis (molting), and therefore they are often referred to as "molting hormones". Ecdysteroids are also involved in other critical physiological processes, including gametogenesis, neurogenesis, growth, and diapause. At the cellular level, ecdysteroids control the process of transcription of specific genes in the nucleus. The precursors for all steroid hormones, including the ecdysteroids, are sterols like cholesterol. Interestingly, insects, unlike mammals and plants, are unable to synthesize the sterol nucleus, and therefore require an exogenous source of sterol for normal growth, development and reproduction. Most (but not all) plant-feeding insects fulfill their sterol requirement by converting dietary plant sterols to cholesterol. Ecdysteroid research directed at pest control is based on the understanding that (1) interference with the conversion of plant sterols to cholesterol or (2) disruption of ecdysteroid biosynthesis and metabolism or (3) interference with hormone action would form the basis of a selective and specific form of insect control.

Basic research on sterols and ecdysteroids is ongoing at universities and in government laboratories; in industry, research usually involves screening programs designed to uncover candidate insect insecticides. Attempts to isolate ecdysteroid receptors has been carried out mostly in academia, both in the United States and abroad. Industry scientists have recently discovered a nonsteroidal ecdysteroid agonist that is active both *in vitro* and *in vivo*, and other research organizations, including ARS, are pursuing this new area. The role of ARS in the elucidation of the biochemical pathways involved in the conversion of phytosterols to cholesterol, and the metabolism of ecdysteroids - including the enzyme systems involved - has been preeminent. A noteworthy strength of ARS has always been its strong analytical capability, and ARS has maintained its dominant position in the area of isolation and physico-chemical identification of ecdysteroids, including their conjugates and other metabolites.

Future Directions

Research thrusts involving ecdysteroids can be broken down into several areas, including (1) sterol utilization, (2) ecdysteroid biosynthesis and metabolism, (3) isolation and identification of ecdysteroids and (4) disruption of insect development. Research directed at the utilization of dietary plant sterols by insects will focus on both pestiferous and beneficial insects (comparative sterol metabolism) in an effort to uncover the extent to which specific phytosterols are precursors for ecdysteroid biosynthesis. Since the conversion of dietary plant sterols to cholesterol is essential for the lepidopteran pests of agriculture, interference with this conversion would constitute a specific form of insect control. The development of specific cell lines from insects incapable of converting phytosterols to cholesterol would facilitate this research program by permitting direct comparison with established lepidopteran cell lines. *In vitro* studies would also complement the search for inhibitors of the various enzyme systems involved in phytosterol dealkylation. Since all insects are dependent on dietary sterols, the feasibility of genetically altering the sterol composition of crop plants will also be pursued under this thrust. Research directed at ecdysteroid biosynthesis and metabolism will concentrate on the characterization of the specific enzyme systems involved in the bioconversion of hormone precursors to active hormone, and biodegradation of the active hormone to inactive metabolites. The isolation and identification of ecdysteroid receptors is a continuing goal. It is known that ecdysteroids receptors differ from peptide receptors in that they are found in the cytosol and are not located on the cell membrane. The disruption of critical physiological processes (e.g. molting, diapause) that are regulated by ecdysteroids will most likely progress from basic research toward application, facilitated by the discovery of structurally simpler, non-steroidal ecdysteroid agonists. This work will be directed at the major lepidopteran pests of agriculture. ARS is significantly involved in the majority of this research, the only exception being the receptor work, which is being conducted primarily by university scientists. Paramount to the research efforts described above is the maintenance and support of analytical instrumentation necessary for the unequivocal identification of ecdysteroids, their precursors, conjugates and metabolites. In the United States, this remains the sole domain of ARS.

These research thrusts are of prime importance. The fact that all insects depend on a dietary source of sterol for normal growth, development and reproduction is the only known nutritional difference between insects and higher animals. Research aimed at exploiting this fundamental difference in an effort to discover new, selective, and environmentally-sound agents for insect control is the driving force behind ARS ecdysteroid research.

Ecdysteroids

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Sterol utilization.	Isolation of sterols, including 24-alkyl sterols from beneficial and pestiferous insect species. (JAS, MFF)	Establishment of cell lines from insects incapable of dealkylating plant sterols. (JAS, MFF)	*In vivo* and *in vitro* biosynthesis studies using radio labeled sterols (JAS, MFF)	Test inhibitors of Δ-24 reductase system *in vitro*. (JAS, MFF)	Test inhibitors of honey bee parasites. (JAS, MFF)
	Determine levels of unusual 4-methyl and 4,4-dimethylsterols tolerated in artificial diets by insects. (JAS, MFF)	Isolate and purify suitable rate-limiting enzyme(s) from corn. (JAS, MFF)	Clone appropriate genes to aid study of their expression in plants. (JAS, MFF)	Modulate specific gene expression to alter sterol profile of plant. (JAS, MFF)	Determine agronomic acceptability of the genetically altered plants. (JAS, MFF)
2. Ecdysteroid Biosynthesis and Metabolism.	Isolate 3-oxoecdysteroid 3α-reductase from *M. sexta* midgut. (GFW)	Determine ecdysone 20-monooxygenase activities in mitochondria and microsomes at various times of development. (GFW)	Characterize ecdysteroid phosphotransferase in midgut. (GFW)	Prepare individual phosphoconjugates for structural identification. (GFW)	Fractionate midgut cytosol to separate phosphotransferases. (GFW)
3. Disruption of Insect Development.	Determine endocrine and neuroendocrine factors involved in embryonic development and diapause. (RAB)	Establish ecdysteroid and PTTH titers during development and diapause. (RAB)	Determine effects and investigate use of hormone agonists on diapause disruption. (RAB)	Refine methods for practical disruption of diapause in laboratory colonies. (RAB)	Conduct small scale field trials against agricultural pests. (RAB)

Ecdysteroids Cont.

4. Isolation and identification (analytical).	The maintenance of state-of-the-art analytical equipment is necessary for the successes of all research programs. Continually refine high performance liquid chromatography (HPLC) and mass spec. (MS) techniques for the isolation and identification of ecdysteroids, hormone precursors, conjugates and metabolites Develop electrospray and HPLC capabilities for use with tandem mass spectrometer. (WRL, MFF, GFW) Collaborate with other scientists in the field to identify bioactive natural products. (WRL, MFF, GFW)

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STEROIDOGENIC NEUROPEPTIDES

Current Status

Steroidogenic neuropeptides induce ecdysteroid production in a variety of tissues and generally fall within two molecular weight ranges, 1-6 kD (small) and 11-30 kD (large). The ecdysteroids are involved in molting, morphogenesis, diapause termination, spermatogenesis, oogenesis, and genital tract maturation, depending on the species of insect and the developmental stage. Large PTTH stimulates the prothoracic glands in Lepidoptera to produce ecdysone or 3-dehydroecdysone that is converted to a biologically active 20-hydroecdysone, the hormone responsible for molting and pupal development. Ishizaki's group has isolated PTTH (MW=30kD) from *Bombyx mori*, sequenced it, cloned it, and located its site of production within the brain and release site from the CA. The gene was cloned and cDNA was introduced into E. coli which produced PTTH. *Bombyx* large PTTH is first synthesized as a 224 amino acid polypeptide precursor that contains 3 proteolytic cleavage signals. A single PTTH subunit contains 109 amino acids. Two units are connected together by disulfide bonds before cleavage from prepro-PTTH to form homo-dimeric PTTH.

Large PTTH has been extracted and characterized from *Manduca sexta, Lymantria dispar*, and *Drosophila melanogaster.* The *L. dispar* gene has been partially sequenced by ARS workers. PTTH activity has also been extracted from *Aedes*, *Calliphora*, and *Sarcophaga*, but no purification or size separation has been done. Using Bombyx PTTH antibodies, PTTH immunoreactivity has been localized to two pairs of lateral neurosecretory cells in the brain protocerebrum of *Bombyx, Manduca, Galleria,* and numerous dorsomedial neurosecretory cells in adult *Locusta.*

Small PTTH - bombyxins - typically have a molecular mass of around 4 kD. Bombyxin is inactive in *Bombyx* but causes debrained *Samia* pupae to develop and the PG to produce ecdysone. Five classes of bombyxins have been isolatedand two have been sequenced. Bombyxins consist of two non-identical peptide chains, A with 20 amino acids and B with 28 amino acids. A and B are held together by disulfide bonds and show a 40% sequence homology with vertebrate insulin. Six clustered genes which encode precursor molecules for peptides related to bombyxin have been cloned. Bombyxin genes have been expressed in *E. coli* and yeast and bombyxins II and IV have been synthesized in greater than 60% yield. Bombyxin-like material or activity has been located in *Galleria, Philosamia*, *Lymantria*, *Manduca, Pieris*, *Rhodnius, Drosophila* and *Locusta.* Small PTTH from *Lymantria* has had a specific nucleotide sequence amplified from cDNA by ARS scientists.

PTTH activity has been isolated from developing ovaries or embryonated eggs in *Lymantria, Manduca,* and *Bombyx.* The function of this PTTH is unknown. ARS workers were the first to extract PTTHfrom the proctodaeum of *Ostrinia* and *Lymantria* and showed that PGs incubated with this PTTH produced ecdysone and 3-dehydroecdysone *in vitro*. This ecdysiotropin(s) has a mass of only 0.5 to 1.0 kD, much smaller than any of the PTTHs found in the brain and may have a role in diapause termination. Other PTTH-like material have been extracted by ARS scientists from the anal papilla of *Lymantria* and causes the PG to produce ecdysteroids *in vitro*. Molecular weights of these ecdysiotropins range between 0.5-1.5 kD and 2-2.5 kD.

ARS scientists were the first to show that a testis ecdysiotropin (TE) is produced in the brain of *Heliothis virescens* and *Lymantria* and induces the testis to produce ecdysteroid. This ecdysteroid then causes the testis sheath to secrete peptidic and lipoproteinaceous growth factors which are required for the formation of the genital tract. In the absence of testis sheath, ecdysteroid induces fat body to produce affective growth factors. TE has been over 90% sequenced and appears to be a unique peptide and that is not related to either big or small PTTH.

EDNH (egg development neurosecretory hormone) is produced within the brains of Diptera and causes the ovaries to produce ecdysone that is necessary for the production of vitellogenin. ARS scientists are the only ones currently working with EDNH. EDNH has been partially purified for *Musca* and has been purified for *Aedes* and partially sequenced. *Aedes* contains multiple forms of EDNH that have a synergistic affect *in vivo*.

Future Directions

The future research directions for steroidogenic neuropeptides will follow a general pattern: determine the amino acid sequence for the different peptides, in *Lymantria, Ostrinia, Heliothis, Aedes, Musca,* etc.. Identify the active core, synthesize peptidomimetics, determine mode of action, locate, isolate and clone genes, insert them into transmission vectors, build antibodies to peptides, locate sites of production by immunocytochemistry and determine peptide titers by RIA/ELISA. Characterize enzymes involved in the synthesis and degradation of the peptides.

Removing PTTH and other ecdysiotropins from the insect system (e.g. inhibition of synthesis, processing; stimulate enzymatic degradation; blocking receptors) would result in ecdysone-deficient insects. Such insects would not molt, and would die as young larvae. Inhibiting EDNH or TE action would result in sterile insects.

Steroidogenic Neuropeptides

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. EDNH (*Aedes* and *Musca*).					
Isolation and Charactirzation.	Sequence EDNH-A ; isolate EDNH-M. (EPM, TSA, TJK, RMW, WRL)	Purify EDNH-M . (EPM, TJK, TSA, RMW, WRL)	Sequence EDNH-M. (EPM, TJK, TSA, RMW, WRL)		
Structure and Function.		Construct and test analogs. (EPM, TJK, RMW, JPK)	MOA studies. (EPM, TJK, RMW, JPK)	Construct binding inhibitors. (EPM, TJK, RMW,JPK)	Test analogs/inhibitors for control. (EPM, TJK, RMW, JPK)
Molecular Biology.	Locate EDNH-A gene. (EPM, TJK)	Isolate EDNH-A gene (EPM, TJK)	Sequence gene (EPM, TJK)	Insert into vector (EPM, TJK)	Test expressed EDNH-A *in vivo* and *in vivo* bioassay systems (EPM, TJK)
Processing, Catabolic Enzymes.	Develop methods and assays. (EPM, RMW)	Characterize enzymes, study kinetics. (EPM, RMW)	Role of enzymes in processing catabolism. (EPM, RMW)	Study enzyme inhibition. (EPM, RMW)	Feasibility in insect control. (EPM, RMW)
Immunology.	Produce antibodies to EDNH-A (EPM, TJK)	Develop RIA, ELISA; Titer peptides; Make antibodies to EDNH-M (TSA, EPM, TJK)	Locate synthesis and release of EDNH with immunocytochemistry (TSA, EPM, TJK)		

Steroidogenic Neuropeptides Cont.

2. Gypsy moth PTTHs (egg and larval).					
Isolation and Characterization.	Isolate PTTHs. (TJK, EPM, RMW, WRL)	Purify PTTHs. (TJK, EPM, RMW, WRL)	Sequence PTTHs. (TJK, EPM, RMW, WRL)	Characterize activity *in vitro* and *in vivo*. (TJK, EPM, RMW, WRL)	
Structure and Function.			Construct and test analogs. (TJK, EPM JPK, RMW)	Construct binding inhibitors. (TJK, EPM JPK, RMW)	Test analogs/inhibitors for control. (TJK, EPM) Isolate receptor (TJK, EPM, RMW, JPK).
Molecular Biology.	Develop PCR screen for PTTH gene. (TJK, EPM)	Isolate gene using *Bombyx* PTTH primers. (TJK, EPM)	Insert into vector, observe expression. (TJK, EPM)	Test expressed PTTH compare with natural PTTH. (TJK, EPM)	Characterize region essential for bioactivity by site directed mutagenesis. (TJK, EPM)
Processing, Catabolic Enzymes.	Develop methods and assays. (EPM, RMW, JPK)	Characterize enzymes; study kinetics. (EPM, RMW, JPK)	Role of enzymes in processing catabolism. (EPM, RMW, JPK)	Study enzyme inhibition. (EPM, RMW, JPK)	Feasibility in insect control.. (EPM, RMW, JPK)
Immunology				Produce antibodies to PTTH. (EPM, TJK)	Develop RIA, ELISA, titer peptides. (EPM, TJK)

Steroidogenic Neuropeptides Cont.

3. Hind gut ecdysiotropin (HGE).					
Isolate, characterize, produce antibodies.	Isolate, purify HGE. (DBG, RMW, JPK, WRL)	Sequence HGE, produce antibodies. (DBG, RMW, JPK, WRL)	Locate sites of synthesis release. (DBG, RMW, JPK, WRL)	Isolate receptors. (DBG, RMW, JPK, WRL)	Design analogs to interfere with HGE action. (DBG, RMW, JPK, WRL)
Physiology of HGE.	Diurnal activity of HGE. (DBG)	Culture *in vitro* to study release. (DBG)	Interaction with other endocrines for synthesis/release. (DBG)	Continue mode of action and interaction studies. (DBG)	
4. Testis ecdysiotropin (TE).					
Isolation and Characterization.	Isolate TEs, Sequence. (MJL, RMW, JPK, WRL)	Structure analysis, solid state synthesis, physiology of action. (MJL, RMW, JPK, WRL)	Solid state synthesis, structure activity studies, cell metabolism, messengers. (MJL, RMW, JPK, WRL)	Construct analogs and develop TE inhibitory analog. (MJL, RMW, JPK, WRL)	
Immunology.			Produce antibodies from synthetic TE. (MJL, SMM)	Develop RIA, ELISA, titer TE.. (MJL, SMM)	
Molecular Biology.					Prepare cDNA library, probe with TE antibodies to isolate TE gene. (MJL)

Steroidogenic Neuropeptides Cont.

TE induced growth factors, GF.	Isolate GF, determine properties. (MJL)	Synthesize GF, mode of action, second messenger. (MJL)	Prepare antibodies, structure activity studies. (MJL)	Probe cDNA library with antibodies from mammalian GF to locate gene. (MJL)	Continue molecular genetics studies. (MJL)
5. Anal papilla ecdysiotropins (APETS).					
Isolation and Characterization.	Isolate APETS. (EPM, DBG, TJK)		Sequence APETS. (EPM, DBG, TJK)		
Structure and Function.				Construct and test analogs of APETS. (EPM, DBG, TJK, RMW, JPK)	Examine analogs and inhibitors as control agents. (EPM, DBG, TJK, RMW, JPK)
Molecular Biology.				Locate APET genes. (EPM, DBG, TJK)	Insert APET genes into baculovirus. (EPM, DBG, TJK)
Processing, Catabolic Enzymes.	Develop methods and assays. (EPM, RMW, JPK)	Characterize enzymes, study kinetics. (EPM, RMW, JPK)	Role of enzymes in processing catabolism. (EPM, RMW, JPK)	Study enzyme inhibition. (EPM, RMW, JPK)	Feasibility in insect control.. (EPM, RMW, JPK)

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ENDOGENOUS REGULATION OF SEX PHEROMONE PRODUCTION IN INSECTS

Historical Perspectives and Current Status

Sex pheromones, which are of critical importance for mating in most insects have been used for both population monitoring and direct control of pest insects. The neural, neurohormonal and hormonal regulators which control sex pheromone mediated-biology provide another significant opportunity for manipulating the mating process of insects. For example, development of methods to inhibit or alter biosynthesis of sex pheromones based on these endogenous regulators may provide highly effective species specific pest management strategies as alternatives to classical control with pesticides.

Research conducted to date has indicated that the endogenous factors which regulate the induction of pheromone biosynthesis include juvenile hormones, ecdysteroids, neuropeptides and biogenic amines. Researchers in ARS, particularly at Beltsville, College Station and Gainesville are leaders in the area of PBAN research. In fact, ARS researchers at the Beltsville facility were the first to document the existence of a neuropeptide regulator of pheromone production in a moth which was subsequently identified from the corn earworm moth and named pheromone biosynthesis activating neuropeptide (PBAN). Their research on structure activity relationships using PBAN analogs led to determination that the C-terminal pentapeptide fragment is the minimal sequence required for activity and identification of a superagonist portion of the PBAN molecule. Other research has led to the isolation and characterization of the gene for PBAN and the fact that the gene carries two other pyrokinin-like peptides. The gene has been successfully inserted into a baculovirus and expression obtained. With the help of antibodies raised against PBAN, they were also able to localize the production and release sites for this neuropeptide as well as develop specific ELISA and RIA. Research at Beltsville led to discovery of a novel PBAN carrier protein in the central nervous system. They also identified host plant volatiles which regulate the production of pheromone presumably by causing release of PBAN. Additionally, a pheromonotropic neuropeptide has been isolated and identified from the gypsy moth. The mode of action of PBAN appears to be different in this species. Research conducted at the College Station facility has focused on structure activity relationships between PBAN and other peptides of the pyrokinin family. This research has shown that the C-terminal pentapeptide fragment, common to all pyrokinins including PBAN, is required for biological activity and that all members of this group elicit significant pheromonotropic, myotropic, and diapause induction activities in appropriate bioassays. One locust pyrokinin (LOM-MT-II) demonstrated superagonist activity in a silkworm pheromonotropic assay by eliciting 100 fold more activity than PBAN itself. Spectroscopic and computer modeling analyses of a rigid, conformationally-restricted pyrokinin, or PBAN-like analog, that retains both myotropic and pheromonotropic activity has shed light on the active conformation adopted by this peptide family at the receptor level. Research at the Gainesville facility has focused on the role of the central nervous system in regulation of pheromone production. These studies have shown that the sex pheromone glands of the corn earworm and tobacco budworm moths are innervated by neurosecretory cells extending from the terminal abdominal ganglion and that electrical stimulation of the terminal ganglion will stimulate pheromone production as will injection of octopamine. Studies have also resulted in the isolation of PBANs from both the brain and terminal abdominal ganglion of the tobacco budworm moth. Ongoing studies at this facility are using PBAN as a tool to probe the mechanisms involved in pheromone biosynthesis in moth species and the interactions between biogenic amines and PBAN that are involved in induction of pheromone biosynthesis.

Researchers in ARS are also at the forefront of studies on the endogenous substances that inhibit production of sex pheromones. Studies conducted in Beltsville documented, for the first time in moths, that factors from the male accessory glands transferred during mating cause the depletion of sex pheromone in females. These studies have led recently to the identification of a 57 amino acid peptide termed "pheromonostatic peptide" from the accessory glands of males of the corn earworm moth that may render the mated female unattractive to additional males. Researchers at the Gainesville facility were first to show a correlation among age, oviposition and reduction in pheromone production in virgin females of a moth species. These studies have led to the discovery of a factor produced by senescing virgins which counteracts the action of PBAN and inhibits pheromone production.

Future Directions

Clearly, significant progress has been made towards elucidating the neurobiological mechanisms regulating pheromone production in a small number of moth species. However, we have only limited knowledge of how pests belonging to other orders control these processes. Consequently, there is a definite need to expand these basic studies to include other insect pests. Ongoing structure activity studies have provided significant information useful for the future design of nonpeptide PBAN agonists and antagonists. The identification of PBAN receptors will greatly aid the design of such compounds. Similar opportunities exist for development of nonpeptide agonists of pheromone suppression factors. Molecular biological studies aimed at identification of the DNA sequences which regulate the titers of pheromonotropic and pheromonostatic peptides and expression of the genes during different developmental stages will be essential to understanding the mode of action of these peptides. Studies on neurotransport proteins and their effects on nerve conduction as well as development of a novel delivery systems will also be of significance for the possible development of novel strategies of pest control

Endogenous Regulation of Sex Phermone Production in Insects

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Neuropeptide (isolation and characterization; molecular genetics.	Begin studies on PBAN gene. (EPM)	Identify PBAN gene. (EPM)			
2. Structure-function studies.	Construct and test analogs of PBAN *in vivo*. (EPM)			Synthesize and test analogs and binding inhibitors; isolate receptors. (EPM)	Explore analogs/inhibitors as control agents. (EPM)
3. Analysis and characterization of processing and catabolic enzymes.	Refine sub-cellular fractionation and membrane preparation methods; develop assays. (EPM)	Characterize enzymes; conduct kinetics studies. (EPM)	Use characterized enzymes in studies on PBAN processing and metabolism. (EPM)	Determine effect of enzyme inhibitors *in vitro* and *in vivo*. (EPM)	Explore enzyme inhibitors as control agents. (EPM)
4. Developmental studies.	Produce anti-PBAN antibody (EPM)	Use anti-PBAN antibody to titer PBAN during development and mate-calling. (EPM)	Initiate studies on PBAN biosynthesis and processing. (EPM)	Continue studies from year 3. (EPM)	Screen for processing inhibitors; identify enzymes involved; synthesize processing inhibitors. (EPM)
5. Structure function and mimetic design.	Synthesis of conformationally-restricted analogs, pseudopeptides, β-turn mimetic systems, and development of active computer peptide surface. (RJN) Prepare antibodies to active, rigid cyclic analogs to serve as receptor models (see Analog-Mimetic section). Probe possible functional implications of the myotropic and pheromonotropic activities demonstrated by PBAN and other pyrokinins. (RJN)			Identification of non-peptide agonist/antagonist candidates from comparisons of computer generated 3-dimensional surfaces of peptide and organic molecules as well as from organic compounds that demonstrate affinity to receptor-model-antibodies. (RJN)	

Endogenous Regulation of Sex Phermone Production in Insects Cont.

6. Design analogs and mimics of PBAN.	Design and test analogs. (AKR)	Design and test mimics. (AKR)	Explore possible ways for using analogs and mimics. (AKR)	Continue exploration for the use of analogs. (AKR)	Evaluate the effectiveness of analogs and mimics. (AKR)
7. Isolate and identify receptors for PBAN.	Prepare and test variously labeled PBAN. (AKR)	Continue receptor binding tests. (AKR)	Isolate and sequence the receptors. (AKR)	Study PBAN/receptor interactions. (AKR)	Explore possibility of interfering with the receptors. (AKR)
8. Elucidate the mode of action of pheromonostatic peptide in *H. zea.*	Isolate and identify the PSP from male accessory glands. (AKR)	Synthesize and determine the mode of action of PSP. (AKR)	Clone PSP gene in *H. zea* specific vector system. (AKR)	Evaluate the effectiveness of the recombinant virus. (AKR)	Continue to evaluate the possible uses for PSP in pest management. (AKR)
9. Study induction and termination of pheromone production in the gypsy moth.	Develop an affective bioassay to test pheromonotropic factors. (AKR)	Study the mechanism through which pheromone production is terminated. (AKR)	Evaluate factors that cause the termination of pheromone production. (AKR)	Explore the possibility of using these factors for pest management. (AKR)	Nothing planned at this time. (AKR)
10. Elucidate plant factors that impact on pheromone production in *H. zea* and *H. virescens.*	Screen known plant volatile from corn, cotton and tobacco. (AKR)	Continue the screening and determine the mode of action. (AKR)	Study the interaction between plant volatiles and PBAN. (AKR)	Investigate possible use of these factors to modify behavior. (AKR)	Continue search for applications. (AKR)
11. Identification of pheromonostatic factors from virgin moths.	Isolation and purification of PBSF from *H. zea*. (PEAT, JHT)	Identification of PBSF. (PEAT, JHT)	Determination of mode of action of PBSF. (PEAT, JHT)	Expand studies to other moth species. (PEAT, JHT)	As in Year 4. (PEAT, JHT)

Endogenous Regulation of Sex Phermone Production in Insects Cont.

12. Identification of pheromonotropic substances from pest Lepidoptera.	Identification of PBANs from brains of *H. virescens*. (PEAT, JHT)	Isolation and Identification of PBANs from terminal abdominal ganglion of *H. virescens*. (PEAT, JHT)	Determination of mode of action of PBANs and If TAG synthesizes PBANs. (PEAT, JHT)	Determination of interaction between PBANs and biogenic amines. (PEAT, JHT)	Expand isolation studies to include other pest Lepidoptera (ex. Plodia, Fall Armyworm). (PEAT, JHT)
13. Identification of neurochemicals regulating pheromone production of Fruit Flies.	Determine chemical/neural mechanisms. Regulating induction of pheromone production in Fruit Flies. (PEAT, JHT)	Isolation and purification of factors inducing pheromone production in Fruit Flies. (PEAT, JHT)	Identification of stimulants. (PEAT, JHT)	Behavioral/Physiologic al studies on inhibition of pheromone production in Fruit Flies. (PEAT, JHT)	Chemical studies of factors inhibiting sex pheromone production in Fruit Flies. (PEAT, JHT)
14. Studies of Biosynthetic Pathways in Key Lepidoptera and Dipteran Pests.	Begin studies to locate sites of pheromone production in each species and identify constituents of glands. (PEAT, JHT)	Continue studies from Year 1. Identify changes in gland constituents with age and diet periodicity. (PEAT, JHT) Determine if neuro hormones/transmitters will induce pheromone production *in vitro*. (PEAT, JHT)	Introduce labeled putative biosynthetic precursors and identify labeled products. (PEAT, JHT) Using pheromonotropic stimulants to induce pheromone biosynthesis. (PEAT, JHT)	Continue with work from Year 3. (PEAT, JHT)	Complete research to determine enzymatic processes that regulate (PEAT, JHT) Biosynthesis and how neural pheromonotropic factors regulate specific biosynthetic steps. (PEAT, JHT)

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NEUROPEPTIDES CONTROLLING WATER AND ION BALANCE

Current Status

The maintenance of a physiologically friendly internal environment is critical to the survival of insects. Whether living in an arid environment where water is scarce and must be conserved or feeding on liquid diets such as blood which contain an excess of water and salts, the composition of insect blood must be maintained within rather narrow parameters. To accomplish this, certain insect tissues have the capacity to selectively remove excess water and specific ions from the blood for excretion. Other tissues can selectively remove ions and water from digestive waste for transport back into the blood as required.

Although it has been known for 30 years that water and ion balance was under endocrine control, it was only six years ago that the first diuretic factors were isolated and structurally characterized by ARS scientists. These eight peptides, the leucokinins, were isolated from cockroaches on the basis of their ability to stimulate visceral muscle contraction. Shortly thereafter, five structurally similar peptides were isolated from the house cricket and subsequently shown to stimulate diuresis in the cricket *in vitro*. Evaluation of the cockroach peptides on mosquito Malpighian tubules (the organs of excretion) demonstrated a positive biological activity and led directly to the isolation of three similar peptides from mosquitoes. An additional peptide of this type was isolated and characterized from locust nerve tissue extracts. All seventeen of these peptides belong to the insect kinin peptide family and were isolated by ARS scientists. The insect kinin peptide family has become a focus of research in structure activity studies, immunocytochemistry, physiology, receptor isolation, and molecular biology at locations within and outside of ARS.

The corticotropin releasing factor-like (CRF-like) peptides are a second group of diuretic neuropeptides present in insects. Six of these rather large neuropeptides have been isolated and structurally characterized (two by ARS scientists) from five insect species. In addition, the gene for one of the CRF-like diuretic peptides has been isolated and sequenced. At the present time, molecular biologists are attempting to clone that gene into a delivery system and express the peptide in an intact insect.

A third class of neuropeptides, the atrial natriuretic-like peptides, has been shown to be present in the stable fly by an ARS scientist. Diuretic and natriuretic effects have been demonstrated on mosquito Malpighian tubules. Studies with ANP-like insect peptides have a high probability of rapid advance since ANP has been a major vertebrate research area for a number of years. Structures of ANP's from several vertebrate sources are known. The gene has been isolated and sequenced and processing of the preprohormone has been described. In addition, two types of ANP receptors have been isolated, structurally characterized, and cloned.

Much less is known about the fourth class of water and ion balance-controlling neuropeptides, the anti-diuretics. This lack of progress is directly related to the difficulty of bioassay procedures. The most recent information suggests that two anti-diuretic neuropeptides are present in the locust. The structural characterization of one of those peptides is approaching completion.

Future Directions

Since water and ion balance are so critical to survival of insects, any scheme which could be devised that would upset that balance has potential as an insect management tool. Over stimulation of diuresis could result in dehydration and death as could inhibition of water conservation mechanisms. Diuresis in insects is a wide-open and rapidly developing research area.

Immunocytochemical studies will be utilized to demonstrate the distribution of diuretic peptides in an insect and determine if they are co-localized. Other immunological techniques will demonstrate release and transport. Physiological studies will determine the cross-reactivity of diuretic peptides from one species on the water balance of other species. Structure-activity studies will show the presence of active core sequences while computer-assisted modeling will lead to synthetic superagonist and antagonist analogs and eventually to a non-peptide mimetic. Receptors will be isolated, characterized, and cloned. Antibodies raised against the receptors will allow the distribution of receptors in the insect to be identified and may suggest other physiological functions besides diuresis. Finally, molecular biological techniques integrated with appropriate delivery systems hold the promise of a new, environmentally sound, and specific insect control technology in the near future.

Neuropeptides Controlling Water and Ion Balance

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Atrial natriuretic peptides in the stable fly.	Isolate the ANP-like peptide from stable flies. (ACC)	Sequence analysis of stable fly ANP-like peptide. (ACC)	Structure/activity studies of ANP-like peptide. (ACC)	Isolation and cloning of ANP-like peptide receptor gene. (ACC)	Continue isolation and cloning of ANP-like peptide receptor. (ACC)
		Identify sites of synthesis, release, and target sites of stable fly ANP-like peptides with immunological techniques. (ACC)			
2. Diuretic effects of Corticotropin Releasing Factor-like and insect Kinin peptides.	Isolate and structurally characterize the CRF-like neuropeptides from the stable fly, salt-marsh mosquito, and tsetse fly (university collaborators). (GMH, FLC)	Evaluate different CRF-like diuretic peptides on several insect species to determine if cross-reactivity exists. (FLC, GMH)	Continue structure activity studies on insect kinin and CRF-like peptides. (RJN, GMH)	Begin isolation and cloning of CRF-like peptide receptors. (GMH)	Express CRF-like peptide receptors. (GMH, RJN)
	Isolate and structurally characterize members of insect kinin diuretic peptide family from house flies, stable flies, mosquitoes, and blowflies (university collaborators). (DLB, GMH, RJN)	Evaluate synthetic analogs as part of continuing structure activity studies on insect kinins and CRF-like peptides. (GMH, RJN, FLC)	Raise and evaluate antibodies against insect kinin and CRF-like peptides of the salt-marsh mosquito. (SMM, FLC)	Reccptor studies (See receptor section). Peptide Mimetic studies (See analog section).	

Neuropeptides Controlling Water and Ion Balance Cont.

3. Anti-diuretic factors.	Demonstrate ADF in diptera and develop bioassay suitable for natural product isolation. (GMH)	Begin isolation of anti-diuretic peptides in stable flies, mosquitoes, and house flies. (GMH)	Structural characterization of anti-diuretic factors from stable flies, mosquitoes, and house flies (university collaborators). (GMH)	Immunocytochemical distribution of anti-diuretic peptide receptors. (SMM) Structure-activity studies on anti-diuretic peptides. (RJN, GMH)	Isolation, structural characterization, and cloning of anti-diuretic peptide receptors (university collaborators). (GMH)
4. Diuretic and antidiuretic factor in *H. zea*.	Isolate diuretic and antidiuretic factors from the ventral nerve cord and corpora cardiaca-corpora allata complexes of *H. zea*. (AKR, MBB)	Confirm biological activity and identify diuretic and antidiuretic factors from *H. zea*. (AKR, MBB)	Synthesize diuretic and antidiuretic factors from *H. zea* and confirm the activity of the synthetic factors. (AKR)	Study impact of diuretic and antidiuretic factors from *H. zea* on reproductive behavior and physiological processes. (AKR)	Evaluate practical applications of *H. zea* diuretic and antidiuretic factors. (AKR)

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INSECT NEUROPEPTIDE ANALOGS, ACTIVE CONFORMATIONS AND MIMETIC DEVELOPMENT

Current Status

Over 70 insect neuropeptide structures have been isolated, sequenced, and synthesized, the great majority of them in the last six years. These peptides have been shown to mediate such critical insect processes as diuresis, visceral muscle contraction, pheromone biosynthesis, ecdysis, diapause induction, etc. The peptides are composed of chains of amino acids held together by relatively unstable "amide" linkages. For this reason, the neuropeptides themselves are susceptible to enzymatic and environmental degradation. In order to disrupt the internal physiological processes maintained by neuropeptides, we must first determine the chemical and 3-dimensional shape requirements encoded in neuropeptide sequences and then utilize this information to facilitate the design of stable pseudopeptide and non-peptide mimics. Mimetics hold potential utility as future specific pest insect management agents.

Progress towards this end has been made with several insect neuropeptide families. The "active core" region, the minimum number of residues required to elicit activity, and critical amino acid residues within the core fragments, have been determined for the pyrokinin (including PBAN), sulfakinin, adipokinetic, leucokinin, and myosuppressin families. Superagonist analogs have been discovered for the pyrokinin, leucokinin, and sulfakinin families in myotropic, pheromonotropic, and "diuretic" assays. The 3-dimensional shape, i.e. "active conformation," that the neuropeptide adopts at the receptor site in order to trigger a physiological response, has been characterized for the pyrokinin and leucokinin families. This was accomplished by synthesis of active, "conformationally-restricted" analogs and the detailed determination of their conformations via a combination of experimental spectroscopic and theoretical computer modeling methodologies. Conformationally-restricted analogs of the pyrokinin and leucokinin families were found to be active in either hindgut/oviduct myotropic, pheromonotropic, and/or "diuretic" assays. Active pseudopeptide analogs, in which one or more of the fragile amide linkages are replaced with stable structures have been synthesized for the leucokinins, sulfakinins, and myosuppressins. For instance, stable "reduced-bond" structures were utilized to replace amide linkages in several leucokinin analogs. For the leucokinin family, the pseudopeptide concept of amide-linkage replacement has been extended to the point where only a single a-amino acid remains. Additionally, a stable chemical replacement has been found for a naturally-occurring but fragile post-translational modification of the sulfakinins. Analogs containing non-peptide C-terminal modifications of the leucokinin and pyrokinin families have been explored, as well. Several inactive peptide analogs have been synthesized that inhibit the "diuretic" activity of the leucokinins and lipid mobilization activity of a dipteran adipokinetic peptide. A bifunctional, heterodimeric analog of the pyrokinin and leucokinin families has been synthesized and has been shown to elicit the otherwise mutually exclusive physiological responses of both families. Each physiological activity elicited by an insect neuropeptide analog represents an additional target (or opportunity) to disrupt the internal environment of a pest insect via a neuropeptide mimetic.

Future Directions

Structure-activity studies should be extended to the newly-discovered classes or families of insect neuropeptides in order to determine active core regions and to identify amino acids critical to activity. Utilizing both spectroscopic and computer modeling methodologies, the characterization of the 3-dimensional shape of conformationally-restricted analogs of new neuropeptide families can shed light on the active conformation required during successful neuropeptide-receptor interaction. Together, information on side-chain chemical and backbone conformational requirements can serve as a guide during the process of designing peptide-mimetic analogs of both new and established insect neuropeptide families. Once the active conformation of a given neuropeptide family is known, the appropriate synthetic turn and/or helix mimetic systems, developed for vertebrate peptides, can be used to prepare rigid peptide-mimetics. The unifying concept of synthetic turn mimetic systems is the replacement of the hydrogen bond with a stable covalent linkage.

The search for pseudopeptide mimetics for established and newly-discovered neuropeptide families should continue, utilizing the variety of available approaches from the vertebrate neuropeptide literature, to replace fragile amide bonds with stable constructs. Metabolic degradation studies of insect neuropeptides can pinpoint specific peptide bonds susceptible to peptidolytic enzyme attack. These sites would become prime targets for the introduction of peptidolytically stable pseudopeptide modifications. New synthetic methods may need to be developed for preparation of these and other peptide mimetics.

Potent neuropeptide mimetics offer the potential to disrupt the delicate internal physiological balance that neuropeptides help maintain in insects. Peptide mimetics can disrupt neuropeptide systems by acting either as a superagonist, whose persistent signal cannot be "turned off" by peptidolytic degradation, or as an antagonist by preventing the neuropeptide signal from interacting with the receptor site. Peptide mimetics may also be important in the characterization of receptor-ligand interaction (i.e. do families featuring different primary structures but elicit similar physiological responses interact with the same or different receptors?). Additionally, some peptide-mimetics may have the capacity to inhibit processing enzymes responsible for either the production of the neuropeptide signal or its destruction once the intended physiological purpose has been met. Receptor binding assays, utilizing isotopically labeled neuropeptide analogs, will be invaluable in future peptide structure-activity studies and in the search for peptide mimetics, at least until receptor proteins can be isolated.

In the future, the discovery of non-peptide mimetics may be enhanced by utilizing computer-assisted modeling/graphics methodologies to screen known macrocyclic organic compounds on the basis of similar chemical structure and 3-dimensional shape characteristics. Alternatively, it is possible that antibodies of potent restricted-conformation analogs, representing receptor models, could be used to screen non-peptide agonist/antagonist candidates for binding affinity. These would represent more biorational (or perhaps "chemorational") approaches to the search for non-peptide mimetics than a purely random screening process.

Following the approaches outlined above, it may indeed be possible to develop future pest insect management agents by utilizing the information encoded in the insect's own chemical messengers.

Insect Neuropeptide Analogs, Active Conformations and Mimetic Development

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Structure-function Studies.	Structure-activity studies on established and newly-discovered insect neuropeptide families. Incorporate receptor binding assays results to complement bioassays. Search for superagonist and antagonist peptide analog. (RJN, JPK, RMW)				
2. Characterization of insect neuropeptide active conformation.	Synthesis of active, conformationally-restricted analogs of established and newly discovered insect neuropeptide families. Characterize conformation of these analogs via spectroscopic and computer modeling methodologies. (RJN, JPK)				
3. Pseudopeptide analog development.	Explore stable isosteric and isoelectronic replacement for peptide bonds, C-terminus and post transcriptional modifications for established and newly discovered neuropeptide families. (RJN, JPK, RMW)			Combine successful replacements together in one or several mimetic systems. Evaluate biological activity. (RJN, JPK, RMW)	
4. Metabolic degradation studies.	Metabolic degradation studies of established insect neuropeptide families. (RMW, RJN, JPK)		Metabolic degradation studies of newly discovered neuropeptide families. (RMW, RJN, JPK)		Apply knowledge from These studies to development of stable mimics. (RMW, RJN, JPK)
5. Non-peptide mimetic Development.	Use information on active conformations to prepare analogs utilizing turn-mimetic systems. (RJN, JPK)			Screen potential non-peptide organic mimetic candidates with antibodies to restricted-conformation (ie receptor models) and with computer- surface. (RJN, JPK)	
6. Receptor isolation and characterization.	Develop active photoaffinity labeled insect neuropeptide analogs. (RJN, JPK, RMW)		Use isolated receptors in analog binding studies. (RJN, GMH, RMW, JPK)		

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PEPTIDE RECEPTORS AND PEPTIDE TRANSPORTER PROTEINS

Definition

Receptors are specific proteins that bind a particular extracellular signaling molecule; the ability of the cell to respond to this molecule depends upon the cell having the receptor. (from Alberts *et al.*). In different target cells the same signaling molecule often affects different proteins and therefore has different effects (*ibid.*). Cells are programmed in two ways: (1) equipped with a distinctive set of receptors for responding to a complementary set of chemical signals and (2) programmed to respond to each signal in their own characteristic way (*ibid.*).

The main purpose for studying receptor5receptors/binding proteins are present and, if so, what are their interactions with neuropeptide as ligands; (2) to develop screening methods utilizing receptors to determine activity of both other neuropeptides and neuropeptide analogs. The substances studied could be derived from non-target species. This technique will probably be more readily available than those based on gene insertion or manipulation.

Current status

In the Agricultural Research Service, studies of receptors for insect neuropeptides have been carried out in three locations. In Beltsville, Livestock Insects Laboratory (LIL), receptors for adipokinetic-type hormones in muscoid flies have been sought. Antibodies have been raised against these proteins. In the Food Animal Protection Research Laboratory (FAPRL) in College Station, receptors for myotropic and diuretic peptides are under investigation. The goal of the Insect Neurobiology and Hormone Laboratory (INHL) in Beltsville is finding receptors for PBAN. In addition to the receptors in cells, the INHL has been conducting research on proteins that bind PBAN and are responsible for its transport in the central nervous system. Receptors for hormones, including peptide hormones, from non-insect sources are of the interest to scientists throughout ARS.

In laboratories outside of ARS, a receptor for insulin was reported and the G-protein receptor gene has been isolated from *Drosophila.* At least one investigator has studied the binding protein for an anti-insect toxin from the scorpion *Androctonus australis*.

The gonadotropin hormone releasing hormone is a small, blocked neuropeptide, somewhat analogous to the AKH family of neuropeptides. There are numerous studies of receptors for this hormone in the medical research community. Receptors for the natriuretic hormone are under study by the medical community. Consideration of the literature on receptors for the opioid peptides suggests that research on these receptors might be of interest to students of insect neuropeptides.

Transport of large molecules such as neuropeptides in the central nervous systems is a new phenomenon, and peptide transporters represent a new class of proteins in insects. For small molecules such as pheromones and juvenile hormones, etc., binding proteins have been extensively studied in insects. In mammalian systems, possible transport of peptides in B-cells for antigen presentation has been proposed, but neuropeptide transport has not been reported.

Future Directions

The scientists in ARS are at various stages of research on receptors. These stages include determination that a receptor is present, isolation, characterization, localization in the insect, partial sequencing, cloning the receptor gene and obtaining the gene product for use in evaluating analogs of the peptide and determining how the receptor can be blocked. It may be possible to introduce a modified gene for the receptor into the fly genome The progeny of these flies would be released in large numbers in a manner analogous to that employed in sterile male release programs.

It could be feasible to modify the insect genome if viruses that could be safely introduced into the medium on which the insect feeds are used as "vectors" or "agents", (see Delivery Systems, this volume). Such modifications might include insertion of genetic material that would code for the active part of the receptor molecule that binds the peptide , that would direct synthesis of excess peptide or would direct synthesis of a peptide that would block the receptor but would not cause normal activity as a result.

In blood-feeding insects affecting livestock, it may be possible to modify the genome of the host so that a gene product interacts with the receptor that is toxic to the ectoparasite is released into the blood of the host animal.

The function of the PBAN transporter protein is somewhat different from receptors in or on cells, but many of the research approaches will be similar. As an approach to "fast action" insect control, in which novel substances will have lethal effects similar to insecticides, insect-specific molecules that interfere with nerve conduction in insects will be designed from the transport studies. These studies on PBAN suggest that the transporter protein for a very different type of neuro transmitter, gamma-amino butyric acid, might be isolated and the findings applied.

Techniques which will be applied during these studies will be high performance liquid chromatography, determination of amino acid composition of both peptide and receptor, affinity chromatography, photoaffinity labeling of the ligand-receptor complex, protein sequencing, molecular cloning of the receptor, determination of the metabolic break-down products of the peptide and its receptor, determination of the 3-dimensional structure of the peptide both by computer modeling of the peptide and studies of interaction of the peptide with its receptor and development of antibodies against the receptor or binding protein. These techniques are general; progress in the research will depend upon the difficulties associated with each peptide and its receptor(s). These techniques are being used by one or more of the laboratories participating in this workshop.

Antibodies will be used against the receptor/binding protein and utilized for tissue localization, isolation of the receptor and determination of the specificity of analogs. Some immunocytochemistry of binding proteins has been attempted, but to date the binding of the antibody has not been very specific.

If the structures of the peptides of interest are known and synthesis is possible, affinity columns can be prepared from the synthetic peptide and used to isolate receptors or binding proteins. Specific antibodies against the peptide can be used in the same manner. The receptor itself, if cloned in sufficient quantity and applied to an affinity column, can be used to isolate identical or very similar peptides from species not previously investigated. This maybe one of the most important early applications of receptor technology.

The mechanisms of interaction, when investigated, can furnish leads in the design of novel materials for population management.

Peptide Receptors and Peptide Transporter Proteins

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Isolation.					
Continue isolation from muscoid flies of receptors for Taa-AKH and Taa-HoTH.	Develop receptor affinity chromatography column to isolate adipokinetic peptides from other muscoid flies. (DKH)	Continue to prepare receptors for muscoid AKH's from natural sources. (DKH)	Continue study of interactions between analogs and muscoid AKH receptors. (DKH)	Continue to isolate receptors against muscoid fly peptides using advances in technology. (DKH)	Finalize results on isolated binding proteins and receptor molecules. (RMW, DJ, AKR)
Isolate receptors for other Taa-AKH or DCC-I type (AKH-type) peptides from muscoid flies.					
Isolate peptides affecting muscoid reproduction.		Continue purification research. Purify receptors for peptides affecting reproduction originally isolated from the reproductive tracts of stable flies, house flies and face flies. (DKH, RMW)	Initiate synthetic program to develop analogs for the reproductive peptide. (RMW)	Continue research. (DKH, RMW)	Finalize results. (DKH, RMW)

Peptide Receptors and Peptide Transporter Proteins Cont.

Determine presence, survey activity and initiate isolation of receptors and binding proteins for myotropic and diuretic peptides.	Develop photoaffinity probe technology for myotropic and diuretic receptors. (GMH, RJN)	Verify isolation of diuretic and myotropic peptide receptors using analogs of these peptides in competitive assays and begin receptor isolation. (GMH, RJN)	Complete isolations. (GMH, RJN)		
Isolate receptor for *Heliothis* pheromone biosynthesis-activating hormone.	Use photoaffinity technique to follow isolation. (AKR)	Continue research. (AKR)	Continue research. (AKR)	Continue research. (AKR)	Finalize results. (AKR)
Isolate transport protein for pheromone activating biosynthesis hormone (PBAN).	Use photoaffinity technique to follow isolation. (AKR)	Continue research. (AKR)	Continue research. (AKR)	Continue research. (AKR)	Finalize results. (AKR)
2. Characterization					
Verify receptor identity; simplify isolation of peptides with similar functions.	Prepare receptor column to isolate related peptides from other tissues; other insect and vertebrate species. (DKH, RMW)	Carry out binding studies and bioassays. (DKH, RMW)	Determine physiological function of muscoid Taa-AKH. (DKH, RMW)	Prepare synthetic muscoid DCC-1 from all species and compare to natural form of neuropeptide for activity. (DKH, RMW).	Finalize results. (DKH, RMW)

Peptide Receptors and Peptide Transporter Proteins Cont.

Verify biological activity of receptors for AKH, myotropic, diuretic and pheromone biosynthesisactivating hormone (PBAN).		Continue research. Determine amino acid sequences, other properties of known and newly isolated muscoid, myotropic, diuretic, and PBAN-like peptides. (RMW, DKH, GMH, RJN, AKR, TBD) Use synthetic and natural peptides to isolate/verify receptors. (RMW, DKH, GMH, RJN, AKR, TBD) (RMW, DKH, GMH, RJN, AKR, TBD)	Determine amino acid sequences and conduct studies to determine protein configuration in collaboration with consultants. (DKH, RMW)	Initiate comparative study of neuropeptide receptors isolated. (DKH, RMW, GMH, RJN)	Finalize data.
3. Immunollogy-Tissue Localization					
Prepare antibodies against AKH-type receptors from muscoid flies.	Prepare polyclonal antibody in rabbit against receptor for Taa-AKH. (DKH, RMW)	Continue purification and localize Taa-AKH receptors using antibodies against the receptor. (DKH, RMW)	Characterize tissue localization (with AB) of receptors. (GMH, RJN, DKH, RMW)	Continue research.	Continue research.

Peptide Receptors and Peptide Transporter Proteins Cont.

Prepare model antibodies against diuretic and myotropic peptides.			Characterize the receptor model antibody for diuretic/myotropic peptides. (GMH, RJN) Begin studying properties of the antibody against the receptors, especially for myotropic and diuretic peptides. (GMH, RJN)	Raise antibodies to the myotropic and diuretic peptide antibodies. (GMH, RJN)	Continue to develop antibodies; consider monoclonal antibodies production. (GMH, RJN)
4. Receptor Metabolism					
Study synthesis and degradation of receptor.	Evaluate presence and absence of receptor before and after binding studies *in vivo* by extractions and other techniques. (RMW, DKH)	Determine possibility of interfering with receptor function by means of receptor analogs. (DKH, RMW) Evaluate technique as insect control strategy by injection or topical treatment with analogs. (DKH, RMW)	Continue studies of year 2. (DKH, RMW)	Begin studies on mechanisms of receptor action in the membrane. (DKH, RMW)	Evaluate feasibility of interfering with receptor "action" in insect control strategies. (DKH, RMW, GMH, RJN, AKR, TBD).

Peptide Receptors and Peptide Transporter Proteins Cont.

5. Molecular Biology				
Determine amino acid sequence and nucleotide sequence of receptor protein.	Clone receptor for Taa-AKH and initiate PCR techniques for sequencing receptor. (RMW, DKH)	Insert receptor gene into microorganisms for mass production of receptor for screening of insecticidal substances. (DKH, RMW)		Insert modified DNA sequences in genome of fly. Purpose is to alter gene so receptor is non-functional. (See item 4). (DKH, RMW) Continue genetic manipulation. (DKH, RMW)
Insert gene into appropriate microorganism to permit batch synthesis.		Determine active gene sequences by studying efficacy of fragments in biological systems. (RMW, DKH)	Sequence the antibody against myotropic and diuretic receptors to obtain DNA code. (GMH, RJN)	Determine potential for carriers to introduce into insect genes that reduce fecundity or viability, possibly using spiroplasmas or viruses, etc. (DKH, RMW, GMH, RJN)
			Introduce genes of non-active peptides into microorganisms. (DKH, RMW)	Determine potential for viral carriers introducing into animals a gene that produces peptide analog deleterious to muscoid flies. (DKH, RMW).

Peptide Receptors and Peptide Transporter Proteins Cont.

Prepare genetic material regulating neuropeptide synthesis for introduction into fly genome. (DKH, RMW, GMH, RJN)

Develop DNA probe based on receptor sequence; isolate gene. (DKH, RMW, GMH, RJN)

Begin preparation of genetic material for laboratory studies for introduction into vertebrate hosts. (DKH, RMW, GMH, RJN).

Synthesize receptors for myotropic and diuretic peptides using fermentation technology. (GMH, RJN)

Peptide Receptors and Peptide Transporter Proteins Cont.

6. Practical Application					
Develop screen for analogs of Taa-AKH (DCC-I) type binding proteins and receptors in flies.	Previously isolated receptor used to evaluate, characterize and localize AKH-like peptides in muscoid flies. (DKH, RMW)	Previously isolated receptor used to evaluate, characterize and localize AKH-like peptides in muscoid flies. (DKH, RMW) Verify isolation using analogs of peptides in competitive assays. (DKH, RMW, GMH, RJN)	Screen non-peptides with receptor model antibody for mimetic potential. (DKH, RMW, GMH, RJN)	Conduct biological testing, including inhibition studies and bioassays. (DKH, RMW, GMH, RJN)	Develop synthetic analogs, determine whether they inhibit growth, survival and reproduction; with commercial firm begin formulation of studies. (DKH, RMW) Complete studies and transfer resources to pilot test for implementation of insect control measures in small scale plots in the field. (DKH, RMW).
Develop screen for myotropic and diuretic peptides.					Develop "off-the-shelf" receptor assay system for leucokinin receptors. (GMH, RJN)

Peptide Receptors and Peptide Transporter Proteins Cont.

Develop screen for PBAN analogs based on PBAN receptor and PBAN transporter protein. Develop technology for modifying receptors.	Use the receptor assay system to evaluate diuretic and myotropic mimetics. (GMH, RJN) Clone antibody genes for the myotropic-diuretic peptide receptors into the delivery system. (For details see appropriate section). (GMH, RJN)

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NEUROPEPTIDE PROCESSING AND METABOLISM

Current Status

The biogenesis of neuropeptides, from gene transcription through secretion of the mature peptide, follows a path which includes mRNA splicing, transport of the prepropolypeptide into the lumen of the rough endoplasmic reticulum, movement of the propolypeptide through the RER into the Golgi, where a variety of processing steps can take place, sorting and distribution of processed peptides into storage and secretory granules, and secretion of the mature peptide. Processing involves proteolytic and non-proteolytic enzymes, and takes place mainly within the Golgi and the subsequent granules. Non-proteolytic processing includes C-terminal amidation and N-erminal pyroglutamate formation, two features common to many insect neuropeptides. The peptidyl alpha amidating monooxygenase (PAM) responsible for C-terminal amidation is the most extensively studied processing enzyme in vertebrates, but has not yet been identified in insects. The advent of available insect neuropeptide sequences has been recent, relative to the situation with vertebrates, and thus there is a knowledge gap between vertebrate and insect systems. However, the gap is smaller than one might assume. The work, for example, of O'Shea and colleagues on AKH precursor biochemistry and proteolytic processing is quite elegant. In addition, only recently have candidate activities (e.g. furin, Kex), which may be true processing proteases, been discovered in vertebrate systems. Strong homology has been found between the genes coding for these vertebrate enzymes, and genes in *Drosophila*. Processing research in ARS (INHL) includes PAM studies and work on the recently published Hez-PBAN gene sequence, which suggests a propeptide structure.

Following secretion and binding of the mature peptide ligand to the receptor, the peptide is cleaved by the action of membrane bound proteases. As with processing, the great majority of information concerning neuropeptide proteolytic cleavage is in the vertebrate literature. However, proteolysis of insect neuropeptides has been studied more extensively than presecretion processing. Endopeptidase and aminopeptidase activities have been reported in *Shistocerca* and *Drosophila* and, in ARS (INHL, LIL), we have discovered and characterized similar activities in *Lymantria* neural tissue. The catabolism of AKH *in vivo* has been reported in *Shistocerca*, and projects to study PBAN catabolism in lepidopterans have been initiated in the INHL. Lysis of AKH/hypotrehalosemic peptides by dipteran hemolymph proteases has been reported by a group from the LIL/INHL/FAPRL.

Future Directions

Characterization of the enzymes responsible for the synthesis, maturation, secretion, inactivation and clearing of neuropeptides should lead directly to the development or discovery of antagonists and superagonists, and the engineering of gene constructs, which can be used to manipulate both the activities of the enzymes, and the production and activities of the resultant neuropeptides. The tools needed for these studies (mature peptide sequences, gene sequences, bioassays) are steadily increasing in number. Much of the technology needed will be the same or similar to that used in receptor work and peptide chemistry, and thus can be shared. The areas which should receive attention are processing, metabolic fate and inactivation. Specific projects are listed as follows:

1. Processing - isolation and characterization of PAM and pGLU-forming enzyme activities (terminal modifications to many insect neuropeptides are essential to biological activity); isolation and characterization of the "furin" gene and its product.

2. Metabolic Fate and Inactivation - tracing specific neuropeptides *in vivo* will identify peptide structural motifs important to the physiological impact of the peptide; characterization of hemolymph and membrane proteases is necessary to understand the control of the neuropeptide signal.

Metabolic fate and inactivation studies will provide useful information sooner than processing studies because of the large database of precedents in the vertebrate literature. However, processing studies are necessary if we are to have the ability to manipulate neuropeptides during both production and action stages.

Neuropeptide Processing and Metabolism

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Neuropeptide isolation and characterization; molecular genetics.	Isolate cerebral PTTH and begin isolation of APET. Fully sequence EDNH(s). Begin studies on PBAN gene. (TJK, EPM)	Sequence PTTH. Isolate APET; begin studies on PTTH and EDNH genes. Identify PBAN gene. (TJK, EPM)	Identify PTTH and/or EDNH gene; insert selected genes into baculovirus system, deduce pre-pro-peptide structures; sequence APET. (TJK, EPM)	Identify APET gene and deduce prepro-structure of neuropeptide. (TJK, JPK, EPM)	Insert APET gene into baculovirus system; use synthetic neuropeptides for expanded physiological studies. (TJK, EPM)
2. Structure-function studies.	Construct and test analogs of PBAN *in vivo*. (TJK, EPM, RMW)	Construct and test analogs of PTTH and EDNH *in vivo* and *in vitro*; start receptor and MOA studies. (TJK, EPM, RMW)	Identify receptors for PBAN and/or PTTH and/or EDNH; continue MOA studies. Construct and test analogs of APET. (TJK, JPK, EPM)	Synthesize and test analogs and binding inhibitors; isolate receptors. (JPK, TJK, EPM, RMW)	Explore analogs/ inhibitors as control agents. (TJK, EPM, RJN)
3. Analysis and characterization of processing and catabolic enzymes.	Refine subcellular fractionation and membrane preparation methods; develop assays. (EPM, RMW)	Characterize enzymes; conduct kinetics studies. (GMH, EPM)	Use characterized enzymes in studies on APET, EDNH, PTTH and PBAN processing and metabolism. (GMH, EPM)	Determine effect of enzyme inhibitors *in vitro* and *in vivo*. (GMH, EPM, RMW)	Explore enzyme inhibitors as control agents. (GMH, EPM, RJN)

Neuropeptide Processing and Metabolism Cont.

4. Developmental studies.	Produce anti-PBAN antibody; develop ELISA and RIA; test analogs of PBAN; produce anti-PTTH and anti-EDNH antibodies. (TJK, EPM)	Use anti-PBAN antibody to titer PBAN during development and mate-calling; use anti-PTTH and anti-EDNH antibodies to titer peptides during development (develop ELISA and RIA). (TJK, EPM)	Initiate studies on EDNH, PTTH and PBAN biosynthesis and processing. (JPK, EPM, RMW)	Continue APET, EDNH, PTTH, and PBAN processing studies. (JPK, EPM, RMW)	Screen for processing inhibitors; identify enzymes involved; synthesize processing inhibitors; examine *in vivo* interaction between PTTH and APET. (JPK, TJK, EPM, RJN, RMW)
5. Neuropeptide Biogenesis and Processing.	Develop and adapt "processing" enzyme assay. (EPM, RMW)	Characterize enzyme activity. (EPM, RMW)	Prepare gene libraries. (EPM)	Isolate enzyme gene(s). (EPM)	Express gene constructs. (EPM)
	Identify enzyme activity. (EPM).	Isolate enzymes. (EPM)	Explore enzyme distribution. (EPM, RMW)	Identify "processing" sites. (EPM, RMW, RJN, JPK) Prepare gene constructs. (EPM)	Examine processing of expressed sequence. (EPM, RMW, RJN, WRL)
	Identify neuropeptide sequences. (EPM, RMW, JPK)		Develop analogs/ enzyme inhibitors. (EPM, RMW, JPK)	Continue analog and inhibitor development. (EPM, RMW, RJN, GMH, WRL)	Test and evaluate analogs/inhibitors. (EPM, RMW, RJN, GMH, WRL)

Neuropeptide Processing and Metabolism Cont.

6. Neuropeptide Catabolism.	Develop and adapt enzyme assays. (EPM, RMW)	Apply assays to natural substrates of interest. (EPM, RMW, JPK)	Begin analog and inhibitor development (EPM, RMW, RJN, GMH, JPK, WRL)	Map enzyme tissue distribution. (EPM, RMW, JPK)	Test and evaluate analogs and inhibitors. (EPM, RMW, JPK, RJN, GMH)
	Develop *in vivo* tracing protocols. (EPM, RMW, JPK)	Collect metabolites and test in appropriate bioassays. (EPM, RMW)	Map metabolite fate. (EPM, RMW, JPK)	Continue analog and inhibitor development. (EPM, RMW, JPK, RJN)	
	Begin enzyme localization and isolation. (EPM, RMW)	Isolate selected enzymes. (EPM)	Produce antibodies against enzymes. (EPM)		

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DELIVERY SYSTEMS

Current Status

During the last decade, there has been an explosion of technology based on recombinant DNA methodology for delivering protein materials to target insects. This explosion continues today as new and improved vectors are created for transforming biological systems ranging from microbials associated with insects to transformation of their own host plants. Little effort is being made by ARS for development of microbial delivery systems. Dougherty (Polydnavirus) and Hackett (Spiroplasma) are developing systems with potential for delivery of foreign gene products to pest insects. All other viral and bacterial systems are being investigated in the academic and commercial arenas. Several groups of micro-organisms are not being investigated for developing recombinant delivery systems for pest insects even though they replicate in the hemocoel and have good potential for control agents. These include insect fungi, protozoa and nematodes. It should be noted that development of prokaryotic delivery systems could only be used on those protein hormones requiring minimal or no post translational modification(s).

Insect viruses, obligate intra-cellular parasites, express gene(s) and their products within host cells where normal and extensive post translational processing occurs. To date, the insect viruses remain the best choice for vectoring protein agents into host insects of economic importance. The Baculovirus expression vector system derived from the Nuclear Polyhedrosis Viruses (NPV) is used routinely. Granulosis Virus (GV) systems are anticipated. Both NPV and GV systems have or will have a variety of weak (capsid gene) and strong (P_{10} gene, Polyhedron gene) promoters. The Entomopox viruses are currently being investigated as expression systems containing both weak (Thymidine kinase) and strong (Spheroidin) promoters. The occluded cytoplasmic Polyhedrosis Viruses (CPV) as well as the large genome containing Iridoviruses also hold potential for future genetic manipulation. The utilization of a variety of virus groups is required due to narrow virus species specificity.

Several groups of bacteria and other prokaryotes (spiroplasma) have potential for vectoring foreign genes. A few species of endophytes have. been used commercially as symbionts (*Rhyzobium spp.*), even as seed treatments. Endophytic bacteria allow the opportunity for the systemic delivery of biopesticides within plant tissue (i.e. the phloem) without modification of the host plant. For example., bacteria from 13 genera have been isolated from *Citrus* xylem. *Erwinia* and *Bacillus* isolates have been recovered from system-free cotton plants that will colonize sterile plants.

Clavibacter xyli subsp. *cynodontis* (Cxc) is a fastidious bacterium found in xylem of asymptomatic Bermuda grass, that was genetically engineered to produce the *Bacillus thurengensis* var. *kurstaki* delta-endotoxin. The commercial product InCide (Crop Genetics International) can be inoculated into corn for control of European corn borer larvae. Data submitted to EPA under experimental use permits (1987-1990) from lab, greenhouse and field studies, showed inoculation of 100+ hybrid varieties of corn seedlings by wounding or pressure-treating of seeds. Cxc/Bti did not survive outside the plant, spread, move in water, overwinter, or survive normal cultivation 6-10 weeks after harvest. There is no secretion of toxin into the plant, so larvae must consume the entire bacterial cell, causing the same symptoms as with Bti. Field tests in 1989 in 4 states showed inoculation effect in some varieties that disappeared during the season: it is not clear that bioactivity was recorded from these fields. Some varieties of corn interacted more favorably than others with Cxc/Bti colonization. Storage of properly inoculated seeds for 1 year is possible, and 1 year shelf life is expected. Advantages include: (1) Environmental safety (2) Farmer convenience (3) minimum disturbance (4) Consistent dosage (5) Season-long protection. (6) Low cost, using a tiny dose per plant, early in season.

The cell wall-less *Mycoplasmas* include *Spiroplasma*, presented in 23 highly diverse groups. *S. citri* are by far the best understood, and have been the subject of molecular technique investigations for 15 years. They carry a small amount of DNA (1000MDa) with a very limited number of genes (ca 200). They are fastidious, need cholesterol and fatty acids for-cell membrane growth, and do not grow at mammalian temperatures of 37^0C. They are found in a number of pest insects (moth, beetles), in many non-pest insects, and it may be possible to use them for delivery of foreign gene products.

Spiroplasma sabaudiense (ex *Ae. vexans,* France, Group XIII), *S. taiwanense* (ex *Cx. tritaeniorhynchus,* Taiwan., Group XXII) and *S. culicicola* (ex *Ae. sollicitans*, New Jersey, Group X) were recovered from mosquitoes. Other isolates including some from *Aedes* (Group XVI) remain uncharacterized: there are doubtless many more in nature. Vectors are needed: no plasmids have been found but extrachromosomal DNA has been described in a survey; some may be useful as vectors. Transformation of *Spiroplasma citri* has been accomplished, but is transient (ca 5 cycles). Note that transient expression or loss of transformed microorganisms during winter or via competition with wild organisms may be an advantage. Transfection of viral SpV1 replicative form (RF) DNA into *S. citri* was recently improved via electroporation, using PEG and CsCl purified DNA. Low efficiency is a problem with usual methods, as it appears that natural competence does not occur in Spiroplasma. Specific procedures are necessary for successful transformation, and will doubtless apply to future attempts in new spp. Transformation of a Colorado potato beetle gut-dwelling Spiroplasma (Group XX) using fibrolin promoter as a vector for a Bti gene is under way (Hackett, Beltsville). Study of cultured *S. sabaudiense* (commercial) and *S. taiwanense* (pathogenic) shows 200 different spots by 2-D gel electrophoresis of proteins and peptides (Humphrey-Smith,, U. Sydney), along with many of the same spots. The challenge here is to identify the source of pathogenicity and to clone the somewhat slower-growing commensal species with the gene generating pathogenicity.

Transformation of the commensal *S. sabaudiense* with a TMOF gene using fibrolin promoter may be possible (Carlson, MAVERL). If transformed, the delivered microorganism should infect the hemolymph of mosquitoes and continuously produce TMOF. Thus, an infected female could not digest a blood meal, becoming effectively sterile. An advantage in using exogenous genes to express small, unblocked peptides as a gene product is that it is so direct as to be possible. A constraint for genetically engineered microorganisms is post-translational processing: in general, it will be more difficult for transformed microorganisms to express modified peptides, such as N-blocked pyro-glu peptides that also require amidation of the C terminus.

TMOF and Related Peptides in Bloodfeeding Insects

Recent accomplishments with the Trypsin-Modulating Oostatic Factor (TMOF) at MAVERL, Gainesville and U Florida, Vero Beach, include:

1. Isolation, sequencing and synthesis completed of the unblocked decapeptide TMOF from mature *Ae. aegypti* eggs. When injected or ingested by blood-fed insects, blood digestion is interrupted, resulting in sterility through loss of egg development.

2. Trypsin biosynthesis is inhibited *in vivo* in 10 spp of mosquitoes, sandflies, stable flies, cat fleas by injection of TMOF and several synthetic analogs.

3. Dose response, time course and ligation lining radiolabelled TMOF suggests degradation by an unknown thoracic factor,, and half life of only 2hr. The metabolite structure is nearly complete. Ligation shows 1000x better activity, now at a few nonagrams (picomoles). Hemolymph-soluble TMOF binds to midgut cells.

4. Partial sequencing of TMOF gene from genomic DNA; complete sequence is expected shortly.

5. Synthetic gene for TMOF in lambda phage successfully transformed 3 *E. coli* strains. DNA was recovered and sequenced to show transformation was successful in the expression vectors. TMOF was produced by all 3 strains by polyclonal RIA and ELISA assay.

6. Cytoimmunochemistry shows production of TMOF by follicular epithelial cells in naturally maturing eggs, as trypsin biosynthesis declines.

Fungi, Protozoa, Rickettsia and Nematodes are all groups of organisms which are either virulent but only under narrow conditions (Fungi) or are highly infectious but are mostly avirulent, (Protozoa). To date, no efforts have been made to develop molecular systems capable of vectoring foreign genes. Plant mycologists have developed transformation and other molecular systems. ARS is initiating a molecular insect fungi program in Ithaca, NY in 1993 to complement classical studies being conducted in several locations within the agency. Production problems of Protozoa, Rickettsia, and Nematodes have dulled interest in these organisms, in the past. Recently, Biosys, a California firm, has mastered large scale (25,000 liter fermentation) production of entomopathogenic nematodes. This breakthrough may lead to interest in genetically engineering of these organisms.

Transgenic plants present one of the most promising means of delivering gene products, however, the product must be; (1) resistant to gut inactivation, (2) active in the gut or (3) capable of transport through the gut to the hemocoel. Plant, transformation of monocots has been easily accomplished in the past via *Agribacterium tumorifaciens* vector derived systems. More recently, dicots have been transformed via physical methods such as electroporation, micro-injection and particle gun technology. Currently, tissue specific promoters for leaves, roots, etc... are being isolated which will allow for differential expression of foreign genes in the host plant thus affording protection to only the desired area of the plant. The major limitation of this promising technology is the stability of the hormone in the gut and its ability to produce a biological effect from the midgut area. An extension of this concept is realized in the efforts of CGI Inc., Hanover, MD. CGI Inc. has used transformed bacterial endophytes (see bacteria) to colonize the xylem of certain cropplants.

Currently, insect transformation efforts resemble the state of plant transformation a decade ago. P-element transformation of *Drosophila* is readily accomplished however, the ability to extend this phenomena even to other Diptera has met with little success. Other genetic elements, including the FLP-FRT yeast system, are being investigated in *Drosophila.* Newer transposons (Tn) from D. melanogaster, HOBO and MARINER, have likewise shown little success in other areas. Tns related to MARINER are widespread in insects. Similar elements are found in plants, suggesting a universal Tn. Work has been in progress with Tn and homeotic genes in *Tribolium* (Beeman, ARS USGMRL, Manhattan, KS and Denell, Kansas State U.) and transformation of lepidoptera using Hsp (Fallon U. Minnesota). The mosquito strain, *A. gambiae*, transformed with Hsp, a neomycin resistance gene, has been maintained since its establishment some years ago at NIH. However, subsequent transformations of injected eggs failed. Transient expression of luciferase/Hsp was done in mosquito embryo using a pneumatic gene gun. Transient expression in fly eggs (Bgal/Hsp) was shown at MAVERL 3 years ago, and there have been no efforts since. It appears that difficulties in transforming insects is the current norm. Transient transformation was shown in *Aedes* mosquitoes, and that was by "cramming in" lots of DNA (Batie, Colorado State u.). It did not work in *Anopheles* spp. which suggests little possibility for general techniques in insect transformation in species more distant then these. Reporter genes used for cloning markers have usually been B-gal or antibiotic resistance genes from bacteria. More useful would be a visible, easily-scored marker such as white-eye, but these genes are not available. Many mobile genetic elements have been "trapped" and partially described in Baculovirus infections of Lepidopteran cells, however, none of these elements have been harnessed to intelligently transform Lepidopteran cells or insects. Recently, Polydnaviruses have been shown to transform Lepidopteran cells in culture. Currently, investigations are underway to determine the feasibility of a Polydnavirus derived vector capable of transforming Lepidopteran pests.

Many insects throughout a broad taxonomic range maintain an intimate relationship with symbiotic bacteria. In some groups of insects this symbiosis bas coevolved into an "endosymbiosis" in which bacteria are housed intracellularly within specialized cells called mycetocytes or bacteriocytes. These mycetocytes frequently aggregate into hundreds of cells forming an organ like structure called a mycetome. These endosymbionts are inherited by offspring through transovarial transmission. Because of their close relationship with the host-insect endosymbionts are characteristically fastidious and have not been successfully cultured. Consequently, the taxonomy and biochemistry of the endosymbionts are largely unknown. Recently, B.C. Campbell. (ARS WRRC, Albany, CA) and colleagues have determined the phylogenetic relationship of the endosymbionts of aphids, whiteflies, mealybugs and weevils to other free-living eubacteria using molecular phylogenetic analysis of the gene encoding 16s ribosomal RNA. The identification of the evolutionary affiliation of these endosymbionts may facilitate efforts to genetically transform the host-insect (and, therefore, its descendants). This transformation could occur through the introduction of new genetic material using the vectors (plasmids) of bacteria which are closely related to the endosymbionts.

The following chart lists some of the potential groups of biological systems capable of delivering hormone products.

Delivery Systems

Delivery systems designed for insect control through endocrine mediation must:
* contain the gene for the neurohormone.
* synthesize the neurohormone.
* deliver the hormone to either the hemocoel, the midgut or both.

The following biological agents/systems target these physiological sites:

HEMOCOEL	MIDGUT
I. INSECT PATHOGENS	
A. VIRUSES	
1. Occluded Viruses	
a. Baculoviruses - Baculoviridae	
i) NPV - Nuclear Polyhedrosis Virus	
ii) GV - Granulosis Virus	
iii) Non-occluded Type C	
b. Poxvirus - Entomopoxvirinae	
	c. Reovirus - Reoviridae - CPV - Cytoplasmic Polyhedrosis Virus
2. Non-Occluded Virus	
a. Picornavirus - Picornaviridae	
b. Parvovirus - Parvoviridae	
c. Rhabdovirus - Rhabdoviridae	
d. Other Viruses	

B. BACTERIA

1. *Bacillus thuringiensis* - gut toxin
2. *Clostridium popillae*
3. Spiroplasma - invasive colonization
4. Endophytes
5. Other Bacteria - Endosymbionts

1. *Bacillus thuringiensis* - gut toxin
2. *Clostridium popillae*
3. Spiroplasma - mid-gut

5. Other Bacteria

C. FUNGI
D. PROTOZOA
E. NEMATODES
F. RICKETTSIA

II. TRANSGENIC PLANTS

A. MONOCOTS - Agrobacterium Mediated Transformation
B. DICOTS - Physical Mediated Transformation

III. INSECT TRANSFORMATION

A. DIPTERA - P-Element and other mobile genetic elements
B. LEPIDOPTERA - Polydnavirus
C. HOMOPTERA, COLEOPTERA - Endosymbionts

Future Directions

Development of microbial delivery systems will be a small effort within ARS in the future. Most laboratories are directed towards development of microbial agents as pesticides and not delivery systems. Academia and industry will most probably develop these systems. However, it is strongly desired that ARS create a nucleus of scientist capable of developing these systems.

There is an active effort within ARS to develop transgenic plants. This work is going on in several locations including Beltsville and Albany, CA. Again, the greatest gains in this area will be made by numerous research teams in academia and industry, who are active in plant transformation for a variety of reasons, including disease resistance, insect protection, herbicide resistance and improvement of food quality.

Insect transformation will remain an area of academic importance. During the next decade, successes will be realized which will improve upon the limited numbers of transforming agents and transformed insects currently available. Currently, insect transformation efforts are too poorly developed for major financial investment. In addition, there is little or no economic payback to industry for anticipated utilization of this technology. In essence, thaese programs would be similar to sterile insect technolgies such as the screw worm program. Thus government and philanthropic organizations will utilize this technology when it becomes feasible. Again, it is deesired that ARS with its strong background in basic biology, pursue certain avenues of research for transforming insect tissue.

Delivery Systems

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Genetic Engineering.	Characterize transform cell lines. Determining what cells can be transformed. (EMD)	Determine if insects can be transformed. Determine if viral integration occurs. (EMD)	Make libraries of formed cells . Determine site of integration. (EMD)	Make vector from knowledge of year 3. (EMD)	Transfer insect tissue with foreign gene. (EMD)
2. Spiroplasma Studies.	Develop vector for spiroplasma transformation. (KJH)	Insect test gene to monitor expression e.g. Beta gal. (KJH)	Insect toxin gene e.g. B.t. toxin for killing insect pests. (KJH)		
3. Genetic Engineering.	Genetic engineering of microorganisms to produce TMOF or modified TMOF. (DAC)				

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DEVELOPMENTAL NEUROBIOLOGY IN TISSUE CULTURE

Current Status

Although the field of tissue culture began in 1907 with the first culture of tissue from the nervous system, and was followed soon afterward by culture of the first insect tissue *in vitro*, there are still no cell lines available from the insect nervous system. At the same time however, progress is being made in the use of dissociated calls from insect nervous tissue.

With respect to insect cell lines in general there are over 400 continuous cell lines that have been reported from more than 100 insect species. This represents a hundredfold increase in cell lines over the three decades since the first insect cell lines were established. While none of these have been proven to be of neural origin, there have been numerous uses of these lines in physiological and developmental investigations. Several cell lines from Diptera, Lepidoptera, Hymenoptera and Coleoptera are responsive to treatment by ecdysteroids, while some cell lines can produce ecdysteroids. Scientists in ARS, particularly at Beltsville, Columbia, Fargo and Gainesville have been leaders in developing new insect cell lines, as well as pioneering in the hormonal studies in tissue culture. Cell lines from diptera are used in genetics research as well, where they are routinely transfected with foreign DNA, while lepidopteran cells are used to express genes for foreign proteins in baculovirus expression vector systems.

Primary culture systems have become important in the investigation of neurite outgrowth, the identification of trophic factors and the role of glial cells in neurogenesis. Insect metamorphosis creates a challenging arena for the interplay of growth factors and cellular morphogenesis. Recent work at the University of Arizona's Division of Neurobiology has focused attention on the use of the developing olfactory system as a model for investigating morphogenesis in the nervous system. This laboratory has been collaborating in the area of tissue culture with ARS scientists.

Future Directions

Clearly, primary cultures can be analyzed biochemically and provide the basis for identifying regulatory and growth factors that operate in shaping the form and function of the insect nervous system. Improvements are needed in the ability to maintain glial cells *in vitro*, and in assaying for trophic factors. The development of cell lines specifically derived from insect nervous tissue is a high priority for this field. Meanwhile, the existing cell lines can be used for examining regulatory mechanisms of neuropeptides, and by transfection producing active peptides in large quantities.

Developmental Neurobiology in Tissue Culture

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Cell and Tissue Culture.	Improve primary cultures of neurons and glia. (HO)	Initiate cell line from immature brains. (HO, DEL)		Identify regulatory factors. (HO, DEL)	

Herb Oberlander (HO)
Dwight E. Lynn (DEL)

IMMUNOCYTOCHEMISTRY

Current Status

With the removal of many insecticides from commercial use and the current focus on biorational insect control, ARS scientists are focusing on biological methods of insect control that target specific insect pests. An approach of this type that appears to have great potential is to disrupt the function of the neurosecretory system of these pests. Studies have shown that secretory products of this system are involved directly or indirectly in the major physiological processes of insects including locomotion, mating, oogenisis, ovoposition, excretion, metamorphosis. By either preventing the synthesis, release or function of these compounds, the life cycle of these insects could be disrupted. In order to achieve control of specific pests species with minimal damage to beneficial insects, it is necessary to identify subsets of the neurosecretory system that can be targeted in specific groups of insects. The most specific process known at this time to accomplish this task exploits the antigen/antibody reaction to determine the presence, distribution, fluctuation and function of neuropeptides specific for a pest species. Realization of this goal should enable the development of synthetic mimics that can disrupt the cycle of these specific peptides.

Within the past seven years a number of scientists throughout the world have become involved in the isolation and characterization of insect neuropeptides. Although ARS scientists comprise a small nuber of these scientists, they have been preeminatnt in this fields. Immunochemistry which uses antibodies produced against these peptides and the idiotypes of their antibodies has become an important tool not only in mapping the distribution of neuropeptides and their receptors, but also in studying physiological and biochemical processes in situ that traditionally required the use of isolated systems. The ability to localize neuropeptides in sectioned material or whole mounts permits the study of prohormones, co-localization of peptides within cells, storage/release sites and the pathways by which these peptides are transported to their target organs. Through experimental manipulation, we can then identify the physiological/biochemical conditions under which these events are promoted or inhibited during various life stages.

The potential for identifying receptor sites in insect tissue with the use of anti-idiotypic antibodies may be critical in determining the function of newly isolated peptides, since the variety of bioassays available is limited. For example, although a large number of myotropic neureopeptides have been isolated using hindgut bioassays, critical, alternate functions may be revealed after mapping the distribution of receptors. Thus the function of the bioassay used to isolate the peptide may not be the primary function of that peptide, rather, multiple functions may exist for a specific peptide, and their expression may be based on titre and the stage of the life cycle of the insect.

Future goals

Technological advances in the past ten years have led to the characterization of numerous peptides from extracts of nervous tissues or in some cases whole bodies of insects. In the later case the tissue producing the peptide is unknown and thus the peptide may or may not be a neuropeptide. Due to the paucity of different types of bioassays and their limits of sensitivity, it has been difficult to determine the pathway by which it reaches its target organ. A solution to these technical problems lies in the use of antibodies raised against synthetic peptides to determine the distribution of the natural peptides in tissues and for quantifying these peptides in tissue extracts. In addition to determining the presence of these peptides in different stages of the life cycle, immunocytochemical studies that include diel periodicity and sexual dimorphism may reveal functions potentially regulated by the neuropeptides. Using computer imaging with brightfield confocal or confocal microscopy, 3-dimensional images of immunoreactive axon terminals in release sites and putative sites of input in the central nervous system will be obtained, leading to an understanding of these peptide chemical messengers. Labeling the antibodies with immunogold will make it possible to determine whether co-localized peptides are packaged in the same neuronal granules or in separate granules. Using ELISA, the same antibodies can later be used to screen for the pressence of newly isolated peptides from other species of insects. An important route to determining the function of specific neuropeptides is to locate their receptors. An approach to this problem is to raise antibodies against the antibodies of the peptides (anti-idiotypic). Because the anti-idiotipic antibodies may image the peptide and bind to the receptor, and since they can readily be coupled to insolvable supports, they can be useful reagents in the purification of receptors. Once the receptors are isolated and purified then antibodies can be made against the receptors. This set of antibodies will be used for DNA sequencing of the active-core to obtain the DNA code. Even partial characterization of the receptors may allow cloning of the gene by conventional methods, providing a simplier route to complete chararcterization. Thus immunochemical studies may complement the efforts and approach taken by ARS neuropeptide chemist. The antibodies produced for these studies will also be usefull in searching for simular receptors in related and diverse insect species. At this time a limited number of insect species have been used for peptide isolation. Immunocytochemistry will be a useful tool in determining the presence of these neuropeptides in other species of agriculturally important insects.

Immunocytochemistry

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Peptide isolation, antibody production and characterization, receptor isolation.	Isolation by HPLC of peptides and sequencing of active peaks. (GMH)	Continue isolation and structural analysis of neuropeptides. (GMH)	Continue immunolocalization at the histological level.. (SMM)	Continue tissue localization studies of newly synthesized neuropeptides. (SMM)	Continue tissue localization studies of newly isolated peptides and quantitation studies in conjunction with variables such as sexual dimorphism, circadian rhythm, and life stage. (SMM)
	Raise antibodies against selected peptides and fully characterize these antibodies with respect to specificity and binding affinity with analogs and ELISA. (FLC)	Continue production of antibodies against synthetic neuropeptides. (FLC)	Continue co-localization studies of neuropeptides at the histological and ultrastructural levels. (SMM)	Continue co-localization and quantitation studies of neuropeptides under experimental conditions. (SMM)	Continue to localize receptor sites in insect tissue using anti-idiotypic antibodies. (SMM)
	Prepare receptor-model antibodies. (FLC)	Continue immunocytochemical mapping to include co-localization of neuropeptides at the histological and ultrastructural level. (SMM)	Quantify the presence of specific neuropeptides by ELISA under different experimental conditions. (FLC)	Localize receptor sites in insect tissues and survey similar receptors in a variety of insects. (SMM)	Isolate and characterize similar peptides and receptors in a variety of insect species in order to determine those that are relatively widespread and those that may be limited to the species requiring control measures. (GMH)

Immunocytochemistry Cont.

	Immunocytochemical mapping of peptides and 3-dimensional mapping of their cell bodies and their neuronal pathways using image analysis and confocal microscopy. (SMM)	Using image analysis with brightfield and confocal microscopy prepare 3-dimensional distributional studies of synthetic peptides and quantify levels of peptide present in cells of origin and storage sites. (SMM)	Characterize receptor-model antibodies. (FLC) Raise anti-idiotypic antibodies against the most promising antibodies and characterize the antibodies using analogs and ELISA. (FLC)

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ROLE OF NEUROREGULATORS IN INSECT CUTICLE FORMATION

Current Status

The Manhattan ARS location has a strong research program in insect cuticle tanning and molting biochemistry, but to date there has been no neurobiology research component in the project. Past work has focused primarily on cuticle structure and metabolism with funding supplemented by USDA and NSF competitive grants. The regulation of cuticle physiology by morphogenetic hormones has been investigated. In addition to ecdysteroids and juvenoids, neuroregulators have been proposed to modulate insect cuticle tanning and molting, but little information is known about the structures, properties and mechanisms of action. Some research bas been conducted in academic laboratories, but little or none in ARS. Studies are needed to characterize more fully the chemical, physical, genetic and regulatory properties of these neuroregulators, and to assess the potential of targeting the tanning and molting neuroendocrine system for insect pest management purposes.

The neurohormone bursicon appears at ecdysis to regulate the conversion of tyrosine to DOPA for sclerotization and melanization of cuticle in species of Lepidoptera and other orders. The tyrosine hydroxylating enzyme (tyrosine hydroxylase or tyrosinase) is somehow activated or substrate accessibility to the enzyme is increased by this polypeptide hormone after its release from the ganglia of the ventral nerve cord. Cyclic AMP and octopamine may also play a role in the process, but little progress has been made in recent years in understanding the mode of action of Bursicon and potential modulators. The puparial tanning factor. a much larger protein than bursicon found in the ganglia of dipterous larvae, plays a similar role in initiating tyrosine hydroxylation through cAMP mediation. Preecdysial tanning and melanization are apparently not regulated by bursicon, but rather are correlated more closely with ecdysteroid and other neurohormonal releases. However, the actual mechanisms are not understood.

As an extension of research on insect cuticle biochemistry, several new biogenic catecholamines, including N-acylated derivatives of dopamine that were originally believed to be unique to the skeletal system, were discovered in the central nervous system of insects by ARS scientists. Although the function of these catecholamines in cuticle sclerotization is fairly well established, their roles in the CNS are unknown. Other biogenic amines, such as octopamine, Dopamine and 5-Hydroxytryptamine, have been detected in the insect CNS by scientists in academic and industrial laboratories. To date these studies only provide useful background information for future work to discern the function and regulation of these factors associated with cuticular tanning and other physiological processes.

Cuticle molting can be induced in epidermal tissues by a primary action of ecdysteroid or by a neuropeptide mediated mechanism. Extracts of abdominal nerve cords from lepidopteran insects stimulate molting enzyme activity, but the neuroregulators responsible have not been identified and characterized.

Future Directions

The metabolism underlying cuticle tanning and molting is relatively insect specific and serves as an excellent target for biorational insect control strategies. We need to extend knowledge of how neuroregulators such as neuropeptides, biogenic catecholamines, neuromodulators and secondary messengers regulate insect development and cuticle formation. Molecular and genetic methods can be used to identify neuropeptides and their genes, as well as biogenic amines and other neuromodulators, that initiate metabolic reactions which eventually cause cuticle tanning and molting. *In vivo* and *in vitro* assays of nervous tissue extracts can be employed to determine effects on tanning and molting enzymes and metabolite levels. The effects of neuroregulators on the activity of various enzyme systems including tyrosine hydroxylases, tyrosinases, N-acyl catecholamine transferases, phenoloxidases, quinone isomerases, chitinases and proteinases can be assessed by kinetic methods. We need to isolate and characterize these neuroendocrine regulators to improve our fundamental scientific knowledge base about insect growth. sThe knowledge obtained would facilitate development of insect growth regulators and biological control agents which disrupt the neuroendocrine control of cuticle formation such that the exoskeleton cannot function as a barrier to biological, chemical and environmental factors which are, hazardous to the pest's survival.

Role of Neuroregulators in Insect Cuticle Formation

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Cuticular Tanning Mechanisms.	Isolate by HPLC neuropeptides and biogenic amines that induce or inhibit cuticle sclerotization or molting. (KJK) Determine morphological, physiological and biochemical activities of these neuroregulators. (KJK)	Determine chemical structures. (KJK) Characterize and sequence tanning and molting neuropeptides. (KJK)	Search for or design compounds such as peptides, pseudopeptides and mimetic compounds that are agonistic or antagonistic to the neuroregulators and that are effective when administered orally, topically or via transformed vectors such as baculoviruses. (KJK) Prepare antibodies and isolate cDNAs of neuropeptide genes. (KJK)	Sequence and characterize cDNAs. (KJK) Develop expression systems. (KJK) Conduct structure-activity-function studies using site-directed mutagenesis. (KJK)	Transform vectors with neuroregulator genes for delivery to insect tissues and for testing as biopesticides. (KJK)

Karl J. Kramer (KJK)

GENETIC AND MOLECULAR ANALYSIS OF NEUROPEPTIDE GENES IN COLEOPTERA

Current Status

The Manhattan ARS location has a strong program in insect genetics with an emphasis on genetic mechanisms of development and pesticide resistance found in the coleopteran *Tribolium castaneum*.. To date there is no neurobiology component in the project. Little is known about beetle neuropeptide genes or more generally about the genetic control of the beetle nervous system development, functioning or sensitivity to pesticides. Genetic studies are needed, particularly in insect taxa containing important pest species such as *Tribolium.*

Classical techniques for chromosome manipulation at the organismal level are used routinely by *Drosophila* biologists to facilitate the study of genetic variation in populations, but such techniques are lacking in other insects. Genetic tools are now being developed by ARS and university scientists to carry out such manipulations in the red flour beetle *T. castaneum* and to apply them to the study of several types of biological problems, including the genetic analysis of insecticide resistance. In principal, these same tools can be used for the genetic analysis of neuropeptide gene structure and function. Artificial mutagenesis can be used to induce neuropeptide gene mutations at a frequency much higher than the spontaneous rate, providing an efficient way to identify genes capable of disrupting neuropeptide function. Progress has been made in (1) constructing balancer chromosomes and other rearrangements which will facilitate chromosome manipulations; (2) mapping the genome with visible genetic markers, recessive lethal mutations and restriction fragment length polymorphisms (RFLPs); and (3) developing a transposon-mediated gene tagging and transformation system. Chromosome manipulations are now possible with *Tribolium* using deletions, duplications and balancer chromosomes to facilitate genetic mapping, dosage analysis, reversion analysis and chromosome extraction, and the prospects for developing the beetle system for germline transformation and transposon-mediated gene tagging and cloning are good.

Future Directions

We need to use genetic and molecular tools to recover mutations in neuropeptide genes in flour beetles, clone such genes, or verify the identity of neuropeptide-like genes cloned by other strategies. We also need to identify RFLPs in neuropeptide genes and map the neuropeptide loci. Chromosome extraction can be employed to obtain viable hypomorphic or lethal null mutations at these loci. Results should help to develop methods that disrupt the genetic control of neuropeptide function.

If the amino acid sequence of a neuropeptide or protein is known, a degenerate probe of the corresponding DNA can be prepared and used to probe Southern blots and identify RFLPs in the *Tribolium* homologue of the neuropeptide gene. To determine chromosome linkage and location, the RFLPs can be mapped in conjunction with visible mutant markers. Mutagenesis and chromosome extraction using a balancer chromosome can be performed to obtain mutations in the neuropeptide gene and to maintain the identity of a chromosomal region that contains a mutant neuropeptide gene during a series of crosses to render the region homozygous. If the mutation is postembryonic lethal, the mutation is recognized by an abnormal phenotype. If it is embryonic lethal, the mutation can be recognized by abnormal immunohistochemical staining or by PCR amplification of the neuropeptide gene from the putative mutant homozygote, followed by restriction enzyme mapping or sequencing. The mutated neuropeptide gene can be identified, cloned and sequenced. The relationship between structure and function of the neuropeptide gene can be studied by the effect of ages in gene structure on the phenotype. If the mutant gene product interferes with function of the wild-type gene product, the mutant gene might have potential for use in insect pest management via transformation of a baculovirus for testing as an insecticidal virus-enhancing peptide.

Genetic and Molecular Analysis of Neuropeptide Genes in Coleoptera

Research Approach	Year 1	Year 2	Year 3	Year 4	Year 5
1. Genetic and Molecular biology techniques.	Develop balancer chromosomes for most of the *Tribolium* genome. (RWB)	Identify interstrain RFLPs in genomic Southern blots using neuropeptide DNA probes. (RWB)	Begin large-scale chromosome extraction's of mutagenized chromosomes, targeting balanced chromosomal regions shown to carry particular neuropeptide genes. (RWB)	Characterize putative neuropeptide mutants, including time of death, nature of behavioral or physiological abnormality, and determine precise map positions. (RWB)	Clone and sequence mutant neuropeptide genes. (RWB)
	Collect DNA probes specific for each of the various neuropeptide sequences. (RWB)	Begin genetic mapping of these RFLPs in conjunction with balancer chromosome; and visible markers. (RWB)			Define limits of stringency for amino acid substitutions, deletions or insertions compatible with neuropeptide function. (RWB)
		Continue to develop new balancer chromosomes. Determine allowable amino acid substitutions in specific neuropeptides by phylogenetic comparisons of amino acid substitutions. (RWB)	Isolate lethal or behavioral mutants in homozygous or balanced form. (RWB)	Screen mutants for abnormal expression by immunohistochemistry. (RWB)	Identify neuropeptide analogs with potential for enhancing virulence of insect viral vectors. (RWB)

Richard W. Beeman (RWB)

RESEARCH RECOMMENDATIONS

A series of recommendations was generated by the participants of the workshop. The goal was to focus on those research areas having the greatest potential for payoff in the near future while at the same time emphasizing those areas where ARS research expertise and experience are strongest with the resources currently available. The areas recommended for emphasis are as follows.

1. Synthesis of neuropeptide analogs and mimetics. This research thrust is designed to produce non-peptide compounds that mimic the action of a neuropeptide but are not inactivated by the proteases of the insect gut or hemolymph. Specific areas to be addressed would include (a) structure activity relationships; (b) computer aided peptide design to obtain three-dimensional structure; (c) mimetic synthesis; (d) evaluation for biological activity; (d) stabilization of analogs and mimetics; and (e) standard toxicological studies.

2. Characterization of neuropeptide receptors. The major goal is to utilize receptors for binding assays required to quickly screen peptide analogs and mimetics for agonist and especially antagonist activities. In addition, antibodies raised against receptor proteins will aid in the discovery of new sites of action in the insect; and genes of receptors cloned into a delivery system offer the possibility of flooding the insect with receptor molecules resulting in hormone imbalance.

3. Molecular biology and genetics. This research area will make major contributions to the neurobiology effort by a) obtaining gene sequences for neuropeptides and their receptors; b) providing delivery systems for genes that disrupt hormonal balance; and c) evaluate anti-sense RNA studies.

4. Assays of biological activity. Physiological and biochemical studies to determine additional phenomena controlled by neuropeptides. Second messenger studies will be emphasized. In addition, similar studies are necessary to determine the mode of action of peptide analogs and mimetics.

5. Regulation of degradation. Neuropeptides are inactivated by specific proteases which may be membrane bound or free in the hemolymph. Compounds designed to specifically inhibit these proteases would have potential as insect management tools.

6. Neurobiology and neurophysiology. This area of emphasis includes a) development of nerve tissue cell lines; b) demonstration and isolation of nerve growth factors; and c) nerve conductance and neurophysiology. Nerve tissue cell lines would provide the physiologists/biochemists with very clean preparations to study neurophysiology, receptor chemistry, and perhaps isolation of new and novel neuropeptides including nerve growth factors.

7. Neuropeptides controlling water and ion balance. Since insects are relatively small and have a high surface to volume ratio, water and ion balance are carefully regulated by several groups of peptides, some of known structural type and others of unknown structure. This thrust would focus on isolation of new diuretic/anti-diuretic peptides, characterization of their receptors, and control of diuresis by factors which stimulate release of the peptides.

8. Increase immunocytochemistry capabilities. Much of the critical background information necessary for rapid advances in other priority research areas (receptors, biological activities, mode of neuropeptide action) can be obtained with appropriate immunocytochemical studies. At the present time, only one full-time scientist is covering this area and cannot cover the quantity of studies requested by other ARS neurobiologists.

9. Mechanisms of neurochemical communication and their effects on diapause, development, and homeostasis. This research thrust would include studies on phenomena such as neuropeptide release, transport mechanisms (both neuronal and humoral) and feedback mechanisms. Information gain from these studies will immediately impact other priority areas such as neuropeptide degradation, synthesis, diuresis, and releasing factors.

All research gaps discussed at the conference have been addressed in the research recommendations and/or 5-year plans have been developed for those areas.

Concerns expressed by scientists at the workshop included a) regulatory; b) industry interest; c) public perception and user acceptance; d) delivery systems; and e) resources.

APPENDIX

The Steering Committee Chairperson and the Steering Committee Members

Dr. Mark Holman, Chair, USDA, ARS, FAPRL: College Station, TX
Dr. Mark Feldlaufer, USDA, ARS, INHL, PSI; Beltsville, MD
Dr. Edward Dougherty, USDA, ARS, INHL, PSI; Beltsville, MD
Dr. Terry Adams, USDA, ARS, RRVARC, BRL; Fargo, ND
Dr. Peter Teal, USDA, ARS, IAL; Gainsville, FL

ARS Insect Neurobiology Workshop
Agenda
Beltsville, MD
October 27-28, 1992

October 27, 1992

PLENARY SESSION

8:30 am	Call to Order -- **Mark Holman** Main Auditorium, Bldg. 003, BARC-West
8:35 am	Welcome and Introduction -- **Robert Faust**
8:45 am	Insect Neurobiology in ARS: An NPS Perspective -- **Ralph Bram**
9:05 am	Workshop Objectives, Charge, and Action Plan Overview -- **Robert Faust**
9:40 am	Adjourn to NAL, Room 1400

TUESDAY MORNING SESSION

Moderator -- **Mark Holman**

10:00 am	Olfactory Sensory Physiology -- **Joseph Dickens**
10:30 am	Open Discussion
10:45 am	Ecdysteroids -- **Mark Feldlaufer**
11:00 am	Ecdysteroid Enzyme Systems -- **Gunter Weirich**
11:15 am	Open Discussion
11:30-1:00	Lunch

TUESDAY AFTERNOON SESSION

Moderator -- **Mark Feldlaufer**

1:00 pm	Steroidogenic Neuropeptides -- **Terry Adams**
1:30 pm	Open Discussion
1:45 pm	PBAN-Historical Perspective, Current and Future Research Trends -- **Ashok Raina**
2:00 pm	Regulators of Pheromotropic and Pheromonostatic Hormones in Insects -- **Peter Teal**
2:15 pm	Open Discussion
2:30 pm	Neuropeptide Control of Water and Ion Balance -- **Mark Holman**
2:45 pm	Atrial-Naturitic Peptides in Insects -- **Andrew Chen**
3:00 pm	Open Discussion
3:15 pm	Break
3:30 pm	Neuropeptide Structure-Activity, Conformations, and Mimetic Analog Development -- **Ronald Nachman**
4:00 pm	Adipokinetic Hormone Family Peptides/Structure-Activity Studies -- **Renee Wagner**
4:15 pm	Open Discussion
4:30 pm	Insect Neuropeptide Receptors -- **Dora Hayes**
4:45 pm	Open Discussion
5:00 pm	Adjourn

October 28, 1992

WEDNESDAY MORNING SESSION

Moderator -- **Peter Teal**

8:30 am	Neuropeptide Processing and Degradation -- **Peter Masler**
8.45 am	Open Discussion
9:00 am	Delivery Systems/Virus Vector Systems & Transgenic Plants -- **Edward Dougherty**
9:20 am	Delivery Systems/Bacterial Vector Systems & Insect Transformation -- **David Carlson**
9:30 am	Open Discussion
9:45 am	Break
10:15 am	Immunocytochemistry -- **Shirlee Meola**
10:30 am	Open Discussion
10:45 am	Insect Tissue and Organ Culture -- **Herbert Oberlander & Dwight Lynn**
11:15 am	Open Discussion
11:30-1:00	Lunch

WEDNESDAY AFTERNOON SESSION

Moderator -- **Terry Adams**

1:00 pm	Research Area/Insect Pest Gaps -- Needs and Priorities

Moderator -- **Edward Dougherty**

2-.30 pm	Program Linkages and Coordination
3:00 pm	Break
3:30 pm	Action Plan Team Meetings -- **Team Leaders**
4:45 pm	Closing Comments -- **Robert Faust, Ralph Bran, Kenneth Vick, Mark Holman**
	Adjourn

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Research Accomplishments

An *in vitro* bioassay has been developed for EDNH (egg development neurosecretory hormone) in *Musca domestica.* This is the only assay available for higher Diptera. *Musca* EDNH has been partially purified and has a molecular weight between 6 and 12 KD.

Research Objectives

Musca EDNH will be isolated and sequenced. An assay for PTTH will be developed and EDNH and PTTH activities will be compared to determine if they are related peptides. Antibodies will be developed to EDNH to determine site of production, release and peptide titer in the hemolymph. The role of the gut and possible EDNH releasing factors will be studied.

1. **Purpose**: To determine the amino acid sequence of EDNH and PTTH.

2. **Significance:** This research will provide the basic information required to construct analogs and peptide mimetic compounds, locate the gene, and isolate the receptor. This information will be used to develop specific insect control technologies that are ecologically sound.

3. **Constraints**: Major constraints at this time are fiscal.

Current and Future Cooperators

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* E. P. Masler, INHL-USDA, ARS, PSI, Bldg. 306, Rm. 309, BARC-East, Beltsville, MD 20705

Potential Uses of Research Findings

Blocking the action of EDNH will result in sterile females. Blocking the action of PTTH would maintain fly and mosquito populations in the larval stage. This would be an ideal control method for Diptera that are pests as adults. Possible means of delivering this technology to a field population depends on the successful construction of peptide mimetics or stable analogs that could permanently block receptors. Such materials could be applied by using conventional delivery systems.

Thoughts on Research Needs

We need more peptide/protein chemists to construct analogs and mimetic materials to be evaluated for insect control.

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Research Accomplishments

1. Determined respiratory activity during embryogenesis, diapause and chill-induced diapause termination in the gypsy moth.

2. Established ecdysteroid and prothoracicotropic hormone (PTTH) titers during embryonic development and diapause.

3. Developed a practical method for prevention and precocious termination of diapause in gypsy moth eggs using KK-42, a novel imidazole terpenoid with anti-juvenile hormone properties.

4. Demonstrated a wide range of developmental effects induced in the gypsy moth by exposure to varying doses of RH 5849, an ecdysteroid agonist, and KK-42, a novel anti-hormone.

Research Objectives

Continue research to develop a comprehensive understanding of the hormonal and neurohormonal mechanisms involved in regulation of late embryonic diapause with particular emphasis on the role of juvenile hormone and the possible involvement of a special inhibitory factor or diapause hormone.

Determine the genetic factors involved in gypsy moth diapause and associated cold-hardiness. Isolate and seek to identify genes and gene products (proteins) that are expressed and associated with diapause.

Explore and develop methods for large scale manipulation of diapause and methods for stockpiling diapausing gypsy moth eggs and selected parasites for biological control programs.

1. **Purpose**: To develop fundamental understanding of the physiological (endocrine and genetic) mechanisms involved in late embryonic diapause and to apply knowledge gained to improve gypsy moth rearing and biocontrol technology.

2. **Significance:** The research is expected to yield new knowledge of the endocrinology and molecular genetics of insect diapause and embryonic development. These studies should also lead to isolation and discovery of novel naturally occurring compounds that will prove useful to development of new and safer pest control agents.

3. **Constraints**: Research is hampered by inadequate heating/cooling systems in the building, lack of proper insectary facilities and difficulty in obtaining good support personnel. Also, cooperation with a molecular biologist will be essential for isolating genes and gene products associated with diapause.

Current and Future Cooperators

Formal cooperation currently in place with LIL, BARC and NASA, Greenbelt, MD and Cape Canaveral FL. Informal cooperation also exists with Al DeMilo, ICEL, BARC; APHIS, Otis ANGB, MA and the Department of Entomology, U Mass, Amherst. Future cooperation with the Entomology Department, U of Maryland and the USFS and Entomology Department at VPI, Blacksburg, VA is contemplated.

Potential Uses of Research Findings

An understanding of the neurohormonal regulation of insect diapause and identification of diapause associated genes and their protein products will facilitate the ability to mass rear and stockpile insect pests and their natural enemies for autocidal and biological control strategies. The goal is to develop the capability to manipulate diapause by preventing its occurrence or inducing, maintaining and precociously terminating it in the laboratory. Research should also lead to isolation of one or more new classes of regulatory peptides/proteins that interfere with growth, development and/or reproduction and therefore pen-nit the synthesis of novel hormonal/antihormonal pesticides.

Thoughts on Research Needs

A team effort dedicated to isolation and identification of hormone/neurohormonal factors and diapause genes and associated gene products is needed for more rapid progress in this area. A molecular biologist (post-doctoral research associate) would be a essential component of such a team effort.

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Research Accomplishments

1. Patents issued on 3 synthetic oostatic hormone (OOSH or Tyrosine Modulating Oostatic Factor, TMOF) analogs of *Ae. aegypti* that show complete inhibition of the proteolytic digestive enzyme tyrosine when injected *in vivo* into 10 species of mosquitoes, biting flies (stable flies, sand flies) and fleas.

2. Discovery of active synthetic TMOF analogs, and also the fate of these peptides by studying longevity of radiolabelled compounds after treatment *in vivo* in mosquitoes.

3. Discovery of an inhibiting factor present in the mosquito thorax that metabolizes OOSH rapidly. Activity with ligated abdomens shown at nanogram level, or 1000x less material than injections into intact mosquitoes, within the range expected for a true hormone.

4. Isolation and partial characterization of the natural oostatic hormone gene of *Aedes aegypti* using techniques of molecular biology.

5. Insertion of a synthetic gene for TMOF into lambda phage, and successful transformation of 3 *E. coli* strains. DNA was recovered and sequenced to show that the desired sequence was in fact present in the transformed expression vector. TMOF was produced by all 3 strains as shown by bioassay, antibody tests, ELISA, and HPLC-MS.

Research Objectives

1. **Purpose**: Working with TMOF and genetically engineered microorganisms, to initiate comprehensive basic studies on selected insects of medical and veterinary importance for the delineation of reproductive processes. Develop toxic or pathogenic organisms through molecular techniques to achieve control of disease-bearing arthropods in nature.

2. **Significance:** Detailed knowledge of the biochemistry of insect peptides may lead to recombinant DNA products and development of new insect control technologies, based on previously unknown and unexploited pathways.

3. **Constraints:** Registration of novel or genetically-engineered materials or microorganisms in the field. Lack of efficacy due to slow growth of novel microorganisms. Lack of appropriate distribution of novel microorganisms in the environment: lack of delivery system. Inability to deliver to the most appropriate locations to achieve control. Inability to produce non-peptidic hormones in plants or microorganisms.

Specific Plans (5 yrs) include:

a. Determination of relevant physiological processes involved in selected species of *Anopheline, Culicene* and *Aedes* mosquitoes, such as interference with the vitellogenic/ oogenic process using TMOF and other aliphatic and steroidal hormones.

b. Genetic engineering of microorganisms such as *E. coli* and *Spiroplasma* species to produce TMOF or modified TMOF for use in the laboratory and eventual deployment in the field.

c. Chemical modification of known TMOF molecules to discover the receptor site and mode of action at the cellular level.

d. Use new knowledge for the development of novel approaches to control of biting or filth-breeding insects of interest, using new knowledge of physiological targets to identify and synthesize molecules to attack these targets.

e. Select appropriate microorganism. for genetic engineering and discover methods for infecting insects in the field.

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Potential Uses of Research Findings

Many important insect species are resistant to pesticides, and although pesticides are disappearing from the market place, replacements are problematic. The materials described herein promise to provide the next generation of pest control materials, particularly as they will be combined with biocontrol techniques. New methods promise to retard development of resistance, and promise species-specificity. Delivery of new technology may be difficult:

1. Field application of generically engineered microorganisms will have to be handled differently from conventional pesticides.

2. The mechanism for transmission /infection of mosquitoes with wild-type pathogenic or commensal Spiroplasma in the field is unknown.

3. Genetically engineered Spiroplasma (GES) (once available) must be infective in the field.

4. Inoculation of plant flowers and extrafloral nectariess with GES may be useful.

5. Production of in vitro cultures of GES is possible.

6. The model for deployment of GES is that of the Mole cricket nematode which carries a pathogenic bacteria, and is presently in successful commercial use in Florida, in bait stations as well as in inundative releases.

Research needs

New equipment for analytical and preparative chemistry, particularly for peptides. Funds for technical personnel are short. Support is needed for interdisciplinary efforts. There is much difficulty in interesting industry in long term efforts, in order to obtain CRADAs to pursue biotech opportunities. More attention should be given to materials that are secreted/excreted from other tissue than the corpora allata. It appears that peptides are produced by non-neural tissue, suggesting that such other tissue is not biosynthetically inert.

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Research Accomplishments

1. Discovered the presence of material(s) immunoreactive to the human atrial natriuretic peptide (ANP) in the stable fly. Demonstrated the diuretic and natriuretic nature of this material(s).
2. Demonstrated the correlation between ecdysteroids and female reproduction in the stable fly.

Research Objectives

Investigate the ANP-immunoreactive material(s) (AIP) for the purpose of potential use of the excretory system as a target for the development of novel insecticides specifically against biting insects.

1. **Purpose**: To understand the hormonal control of the excretory process in biting insects for the exploitation of the system for novel insecticide development.

2. **Significance**: Unlike phytophagous insects, which generally feed continuously, biting insects are intermittent feeders. Feeding turns on diuretic process in biting insects to remove the excess fluid from the sudden influx of blood meal. it is conceivable that control agents developed toward the excretory process would be especially effective against biting insects.

3. **Constraints:** For the rational design of control agents based on peptides, the knowledge of receptors for these peptides is essential. The lack of expertise and facility in this area will hinder the progress.

Current and Future Cooperators

* B.J. Cook, ARS, College Station, TX.
* T.J. Kelly, ARS, Beltsville, MD.
* T. Pannabecker, Cornell University.
* R. M. Wagner, ARS, Beltsville, MD.

Potential Uses of Research Findings

Diuretic hormones appear to be turned on by feeding specifically in bloodsucking insects. When we obtain extensive knowledge on these hormones and how they interact with target tissue(s), it should be possible to devise chemical control agents directed toward the excretory apparatus of these important pests. It is also possible to design the chemicals toward only the bloodsucking insects thus achieving selectivity to pest species. Agents developed specifically for bloodsucking pest could be delivered simply by injection or oral ingestion. If these agents prove to be vulnerable to degradation by the livestock, micro encapsulation into carrier molecules could be used as the delivery system.

Thoughts on Research Needs

More research efforts should be directed to receptors for hormones. in order to develop future chemical control agents for pest insects targeted at peptide hormones, it is imperative that we understand the receptors for these peptides. Only with a thorough knowledge of the receptor would it be possible to take a rational approach in designing potent and selective insecticides against the pest species.

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CRIS #: 6202-32000-003-OOD
Phone: (409) 260-9320
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Research Accomplishments

1. Established the pharmacological actions of the leucokinin neuropeptides on the visceral muscle systems of the cockroach.

2. Determined the basic structural, physiological, and pharmacological properties of key visceral muscles in the reproductive tract of the female stable fly.

3. Developed ultramicrotechniques to detect and measure muscle movement

Research Objectives

Investigate the mode of action of recently discovered neuropeptides in the stable fly and other insects with special emphasis on how they interact with the bioelectric and mechanical events that occur in key muscle groups.

1. **Purpose**: To understand the biochemical mechanisms that regulate such vital processes as oviposition, ovulation, and excretion in biting flies.

2. **Significance**: It is evident that movement is the principle avenue for expressed behavior in insects and such complex functions as oviposition, ovulation, and excretion require a delicate regulation of muscle activity. Consequently, any fruitful attempt to target such functions for control purposes would necessitate a fairly comprehensive knowledge of them.

3. **Constraints**: Any rational development of control agents based on peptides must consider the fact that neuropeptides are multifunctional entities that have broad range of activities on many different physiological systems. A lack of understanding on this matter can hinder progress in the development of such control agents.

Current and Future Cooperators

* A.C. Chen, ARS.
* R.M. Wagner, ARS.

Solving High Priority Pest Problems

The physiological and pharmacological studies outline above will provide the fundamental facts necessary to devise rational chemical control agents that target the reproductive processes of biting flies.

Thoughts on Research Needs

In order to develop future chemical control agents that target peptide hormones, it is necessary that we understand how the specific peptide interacts with other chemical messengers such as neurotransmitter and modulators. Only with such knowledge will it be possible to devise effective agents that have the proper potency and selectivity against pest species.

Areas of Insect Neurobiology Which are Not Being Addressed Within ARS but Have Potential for Breakthroughs

The study of arthropod venoms and toxins have been largely neglected in ARS. Certainly investigations of these agents of admirable design would be most instructive in the development of future control technologies; i.e., Tomalski, M. D. and Miller, L. K. Insect paralysis by baculovirusmediated expression of a mite neurotoxin gene. *Nature* 352:1991.

Name: **Minh-Tam B. Davis**
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CRIS #: 1275-22000-081-OIT
Phone: 301-504-8737
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Research Accomplishments

1. Discovered a 110 kilodalton transporter for PBAN in *Helicoverpa zea.* Transport of big molecules such as peptides in the central nervous system is a new phenomenon, and their study opens a new field of research.

2. Cloned, for the first time in insects, the gene for PBAN in *H. zea.* The results have placed our laboratory in the leadership position in molecular biology of pheromone.

3. Cloned genes that might be involved in postembryonic neurogenesis in *Manduca sexta.*

Research Objectives

* Continue to characterize the PBAN transporter in *H. zea* and eventually clone its gene.
* Study transporters for neurotransmitters, and peptides other than PBAN.
* Identify PBAN receptor(s) in *H. zea* and eventually clone its gene.
* Identify DNA sequence(s) regulating the abundance of PBAN production, identify the site(s) of PBAN production and the expression of PBAN gene during various developmental stages of *H. zea.*

1. **Purpose**: Isolation of genes for the PBAN transporter and receptor(s) will lead to information about their modes of action, their structures and the regulation of their production.

2. **Significance**: Study of genes for transporters and receptors will lead to new approaches to insect control, and provide the ARS leadership position in insect molecular neurobiology.

3. **Constraints**: More manpower, such as lab technicians, is needed since the above-mentioned research areas are highly competitive.

Current and Future Cooperators

* W. Roelofs, Cornell Univ., Geneva, NY
* G. Prestwich, The Center for Biotechnology, SUNY at Stony Brook, NY
* I. Paul, Neurobiology Laboratory, NIH
* V. Vakharia, Center for Agric. Biotech., Univ. of Maryland, College Park, NM
* J. Kochansky, USDA, ARS, INHL, Beltsville, MD
* R. Wagner, USDA, ARS, LIL, Beltsville, MD

Potential Uses of Research Findings

Study of neurotransmitter transporters will lead to a new approach to insect biocontrol, with lethal effects on nerve conduction, similar to insecticides.

Thoughts on Research Needs

Whereas mammalian molecular neurobiology is an exploding forefront research field, insect. molecular neurobiology is not well supported in ARS. Isolation of genes for neuropeptides such as PBAN, ecdysiotropins, diuretic and antidiuretic peptides is needed to study their regulation. Isolation of genes for transporters and receptors of neuropeptides is needed to study their modes of action, their structures and to design their analogs for insect control. All the above-mentioned research areas are highly competitive. Yet, at least in our laboratory (INHL), there are only non-permanent SYs with expertise in molecular neurobiology. With only non-permanent SYs in this area, it is a very unstable situation for ARS to obtain and/or maintain leadership positions.

Name: **Joseph C. Dickens**
Laboratory: Boll Weevil Research Unit
Address: P.O. Box 5367, Mississippi State, MS 39762

CRIS #: 6406-22000-010-OOD
Phone: Com. 601-323-2230;
FTS #: 700-497-1172
FAX: Com. 601-324-1695

Research Accomplishments

1. Characterized olfactory receptor neurons in the boll weevil, *Anthonomus grandis.*

2. Discovered that activation of a specific class of receptor neurons for *green odor* enhanced behavioral responses to pheromones, which were detected by other classes of receptor neurons, in the boll weevil and a number of other insects (patent being processed); found that *green odors* interrupted pheromone responses in insects calling in a monoterpene environment (patent being processed).

3. Elucidated analogs of unstable boll weevil pheromone components which stimulated identified pheromone receptor neurons, and could substitute for the chemically-unstable pheromone components.

4. Determined specificity of receptor sites on identified pheromone receptor neurons through single neuron recordings and designed molecules.

5. Determined relationship between activated receptor neurons and behavior in the boll weevil.

Research Objectives

Investigate mechanisms of olfactory reception of semiochemicals by insects. Provide knowledge of specific types of olfactory receptor neurons in selected insects. Investigate potential agonists or antagonists of receptor sites on identified neurons. Determine effects of receptor neuron activation through laboratory and/or field behavioral tests. Target insects include the boll weevil, *Spodoptera* moth species, parasitoids (e.g. *Microplitis croceipes)* , and selected predaceous and phytophagous Heteroptera (e.g. *Lygus lineolaris).*

1. **Purpose**: Chemical signals utilized by insect pests offer one means for manipulation of insects for crop protection. Chemical signals used by parasites and predators could concentrate beneficial insects in areas where host are located. Information gained in the elucidation of olfactory receptor mechanisms will then be used in designing potential agonists and antagonists of receptor sites on identified neurons.

2. **Significance**: Results of these studies will provide bases for exploitation of insect chemical communication systems for new, environmentally-sound, biorational behavioral manipulation of pestiferous insects. Results will also provide useful insights into chemical communication systems of other insects such as parasitoids as well as basic knowledge of insect chemoreception.

3. **Constraints**: Currently, olfactory sensilla housing receptor neurons for air-borne semiochemicals in some pestiferous species being investigated are small and difficult to record. There is a lack of chemists and other neurobiologists at my location to provide support and cooperative interactions. Additional equipment is needed for new experimental approaches, e.g. patch clamping.

Current and Future Cooperators

* J.R. Aldrich, Insect Chemical Ecology Laboratory, Beltsville, MD;
* W.P. Wergin, Electron Microscope Laboratory, Beltsville, MD;
* D.M. Light, Plant Protection Research, Albany, CA;
* E.B. Jang, Tropical Fruit and Vegetable Research, Hilo. HI;
* G.L Snodgrass, Southern Insect Management Research, Stoneville, MS;
* J.W. Tumlinson, Chemistry Research, Gainesville, FL.
* F.E. Hanson, University of Maryland, Baltimore, MD;
* T.L. Payne, Ohio Agricultural Research and Development Center, Wooster, OH;
* G.D. Prestwich, State University of New York, Stony Brook, NY;
* R.J. O'Connell, Worcester Fndt. for Experimental Biology, Worcester, MA;
* T.A. Christensen, University of Arizona, Tucson, AZ;
* A.M. Hammond and D.P. Pashley, Louisiana State University, Baton Rouge, LA
* S.B. Vinson, Texas A&M University, College Station, TX;
* K. Mori, University of Tokyo, Japan;
* J. N. C. van der Pers, VDP Laboratories, Hilversum, the Netherlands;
* J. H. Visser, Directorate for Agric. Research, Research Institute for Plant Protection, Wageningen, The Netherlands;
* J.H. Borden, Simon Fraser University, Burnaby, B.C., Canada.

Potential Uses of Research Findings

Current research in my laboratory centers on plant bugs including *Lygus lineolaris,* the boll weevil, and Lepidoptera of the genus *Spodoptera.* Results of these studies will provide an understanding of mechanisms involved in reception of chemical signals by these pestiferous insects, and will lead to new or improved attractants, interruptants, or compounds with novel effects on insect behavior. These chemicals will then be used in biorational, economic, environmentally-sound behavioral modification control strategies. Chemical signals may impart species specificity to attracticide devices thus facilitating use of other control strategies, e.g. pathogens or neuropeptides.

Thoughts on Research Needs

Equipment is needed for isolation and characterization of binding sites from olfactory membranes. Research effort could be enhanced by consolidation of related or complementary research efforts at one location.

Name: **M. F. Feldlaufer**
Laboratory: Insect Neurobiology & Hormone Laboratory
Address: Bldg. 467; BARC-East, 10300 Baltimore Ave., Beltsville, MD 20705

CRIS #: 1275-22000-057-OOD
Phone : 301-504-8389
FAX #: 301-504-8526

Research Accomplishments

1. Demonstrated that a lepidopteran continuous cell line was capable of synthesizing 20-hydroxyecdysone.

2. Identification of a 29-carbon ecdysteroid (makisterone C) as an embryonic molting hormone of cotton stainers.

3. Identified makisterone A (24-methyl-20-hydroxyecdysone) as the major molting hormone in the honey bee.

4. Demonstrated that neutral sterols other than cholesterol can serve as precursors for ecdysteroids.

Research Objectives

* Investigate utilization of dietary sterols and ecdysteroid biosynthesis in economically-important insect species (pestiferous and beneficial).
* Establish specific cell lines from insects not capable of converting dietary phytosterols to cholesterol.

1. **Purpose**: To elucidate the biochemical pathways involved in molting hormone biosynthesis and metabolism.

2. **Significance**: To provide environmentally-sound strategies to control insects based on unique aspects of insect biochemistry.

3. **Constraints**: Lack of specific insect cell lines. Lack of a permanent SY in molecular biology.

Current and Future Cooperators

* R. Imberski, University of Maryland, College Park, MD
* E. Bernays, University of Arizona, Tucson, AZ
* D. Wheeler, University of Arizona, Tucson, AZ
* A. Raina, ARS, INHL, Beltsville, MD
* H. Shimanuki, ARS, BRL, Beltsville, MO
* S. Buchmann, ARS, HBIBCRL, Tucson, AZ

Potential Uses of Research Findings

Interference with sterol utilization or molting hormone biosynthesis would form the basis of a control strategy based on a unique difference between insect and mammalian biochemistry. Information may also be useful in controlling parasitic Acari of the beneficial honey bee.

Thoughts on Research Needs

Analytical instrumentation is currently state-of-the-art. It will be necessary to maintain this strong analytical capability to unequivocally identify bioactive compounds and their intermediates.

Name: **Dale B. Gelman**
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Research Accomplishments

1. Discovered the presence of an ecdysiotropin (stimulates the production of an ecdysteroid which is a precursor to the molting hormone) in the hindguts of the gypsy moth and the European corn borer (ECB). Characterized the peptide, determined its 2nd messenger and its interaction with ECB PTTH.

2. Determined that PTTH is present in brains of diapausing ECB and that prothoracic glands from these larvae are refractory to stimulation by PTTH.

3. Discovered and characterized a 3-oxoecdysteroid 3β-reductase (ketoreductase) in the brain, hindgut, testes, salivary glands and other organs of the European corn borer. This enzyme converts 3-dehydroecdysone to ecdysone, a precursor of the lepidopteran molting hormone.

4. As part of a team, discovered that factors in the anal papilla, as well as intact neural connections to the terminal abdominal segment of the gypsy moth, are necessary for larval-larval and larval-pupal molts. Determined that at least one of the above factors is an ecdysiotropin.

Research Objectives

Isolate and purify the ecdysiotropic peptide(s). Sequence the ecdysiotropin(s). Determine the physiological role(s) of the peptide(s). Produce antibodies to the peptide(s) to facilitate its detection so that its distribution in the insect as well as its site of production can be located.

1. **Purpose**: To isolate, characterize and determine the mode of action of newly discovered ecdysiotropic peptides from the insect hindgut and anal papilla (AP).

2. **Significance:** To utilize this knowledge to design and synthesize new agents that will interfere with insect molting and metamorphosis, and thus act to control insect pests.

3. **Constraints**: These peptides are very potent but are present in minuscule amounts in the insect hindgut and AP. Active fractions are not producing a visible peak on the chromatogram. This will necessitate dissecting and processing very large quantities of tissue.

Current and Future Cooperators

* Robert Bell, INHL Beltsville, MD
* Tom Kelly, INHL Beltsville, MD
* Jan Kochansky, INHL Beltsville, MD
* Marcia Loeb, INHL Beltsville, RD
* Bill Lusby, INHL Beltsville, MD
* Pete Masler, INHL Beltsville, RD
* Belgaum Thyagaraja, Dept. of Zoology, Univ. of Maryland
* Renee Wagner, LIL Beltsville, 14D
* Nancy Beckage, Dept. of Entomology, University of California at Riverside
* Karen Meese, Dept. of Entomology, University of Minnesota
* Klaus Richter, Sachsische Akdemie der Wissenschaften zu Leipzig, Jena, FRG

Solving High Priority Pest Problems

Design and implement additional alternative environmentally-safe control strategies for managing insect pest populations.

Thoughts on Research Needs

Technology to rapidly determine the structures of peptides that are present in minuscule quantities.

Name: **Dora K. Hayes and Nita M. Minnifield**
Laboratory: Livestock Insects Laboratory
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CRIS #: 7265-32000-033-OOD
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FAX #: 307-504-9067

Research Accomplishments

1. isolated and characterized receptor from the face fly for a dipteran adipokinetic hormone (AKH).

2. Utilized receptor to evaluate analogs of dipteran AKH.

3. Demonstrated enhanced death rate after treating face fly adults with antibody against the dipteran AKH.

4. Demonstrated slow toxic action against face flies of injected analog of dipteran AKH.

5. Developed technique to study binding to and penetration of peptides through integument of face flies.

Research Objectives

* Continue to isolate, characterize and develop analogs for peptides that affect reproduction and lipid and carbohydrate metabolism in muscoid flies, specifically house, stable, face and horn flies.

* Isolate and characterize receptors for dipteran peptides affecting reproduction and clone gene for receptors as resources become available.

* Produce receptors for one or more dipteran peptides in sufficient quantity so that these proteins can be used to screen analogs of the natural peptides.

* Determine mode(s) of action of reproductive and adipokinetic peptides; use this information to design compounds for use in IPM muscoid fly control programs.
* Determine feasibility of introducing genes directing synthesis of non-active receptor proteins or of non-active reproductive or adipokinetic peptides into insect progeny of which would then be released in a program comparable to the sterile male program.

* Determine feasibility of introducing genes that will direct synthesis by the host animal of molecules that are related to the peptides under study and that block binding of the reproductive or adipokinetic peptides in the pest insects.

1. **Purpose**: Control of insect pests of livestock and poultry.

2. **Significance**: Reduce contamination by other pesticides; develop control methodologies for insects that are resistant to pesticides currently being used, develop components of IPM program.

3. **Constraints**: Budget, lack of technical support, need for increased acceptance of such a program for livestock insects.

Current and Future Cooperators

* T. Coudron, MWA
* D. Gelman, BA
* A. Hung, BA
* B.J. Cook, SPA
* A. Raina, BA
* L. Owens, BA
* A.C. Chen, SPA
* E.P. Masler, BA
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* R. Hammond, BA
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* J. Kochansky, BA
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* K. Kamo, BA
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* R.W. Miller, BA
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* R.M. Wagner, BA
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* C. Rexroad, BA
* L. Johnson, BA
* D. Bolt, BA
* M. Hodkova, Czech. Acad. Sci.
* T. Hodek, CAS
* J. Nelson, U. MD
* M. Ma, U. AD
* B. Fraser, FDA
* J. C. Hill, FDA
* H. Schreier, UMBC
* H. Jaffe, NIH
* T. August, Johns Hopkins
* C. Riley, Johns Hopkins

Potential Uses of Research Findings

Resistance is developing against insecticides in current use against livestock insect ectoparasites. There are few novel insecticides on the horizon; development and registration costs for conventional molecules exceed $43M in most cases. Effective IPM programs consist of more than one component and the research on receptor will lead to development of a novel set of technologies for insect population management.

Technology transfer can occur between ARS scientists and companies that manufacture or produce materials or apparatus that are used in insect population management programs. One such company might be Mycogen. This company can incorporate portions of the genome into bacteria other than those in the genus, *Bacillus*.

End users, such as the farmer, home-owner, military installation or civilian hospital, restaurant, rest-home and government agency (parks, local governments, etc.) could be given dry or wettable powder to dispense with a suitably designed apparatus.

Some materials that would enter the insect an contact might be provided on traps. This could include some of the genetic materials in vectors such as spiroplasmas, viruses, bacteria, etc., as well as materials that would affect the receptor directly, such as analogs of the peptides.

Insect releases, if insects with modified receptors are to be used, could be controlled by the Animal and Plant Health Inspection Service. This would require mass-rearing facilities and details are beyond the scope of this description.

Thoughts on Research Needs

Collaboration among the ARS laboratories that can contribute to solution of a given receptor problem will reduce the need for duplication of expensive and rapidly outdated equipment.

Specific equipment needed will include agarose gel electrophoresis set-ups, DNA synthesis and DNA sequencing equipment and free flow electrophoresis equipment. For most effective development of the methodology, the capability to model structures by computer should be acquired by ARS. ARS also needs to consult with peptide chemists such as Renee Wagner to determine what mass spectrometry equipment best meets the needs of those studying peptide structures of varying molecular weights. A circular dichroism apparatus at each major location (College Station, Beltsville, for example) would also be a necessity.

Areas of Insect Neurobiology Which are Not Being Addressed Within ARS but Have Potential for Breakthroughs

We are beginning to address the genetics and molecular biology of the peptides and their receptors as indicated in this write-up, but the research would proceed more rapidly if the investments of time and money were both increased and more sharply focused.

Insect Pest Species That Might Be Vulnerable to a Neurobiology Approach but at Present are Not Being Studied With That Approach in Mind

Ticks, Lesser mealworms and other beetles, Sand flies,
Culicoides, Black flies,
Tabanidae

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CRIS #: 6202-32000-003-OOD
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Research Accomplishments

1. Directed the isolation and structural characterization of fifteen unique neuropeptides from Locust brains/cc complexes

2. Developed method for isolation of neuropeptides from whole-body extracts of small insects (mosquitos, flies). Six new peptides were characterized from mosquitos and three from stable flies.

3. Developed method for isolation of CRF-like diuretic peptides from whole-body extracts of flies and mosquitos. The CRF-DP of the stable fly has been isolated and sequenced.

Research Objectives

The focus of the next five years will include: a) diuretic and anti-DH peptides in bloodfeeding diptera, structural identification, stimulation of release, transport and mode of action; b) diuretic and anti-DH peptide receptors. For details, see the 5-year plan; c) toxicology of peptide mimetics.

1. **Purpose**: Obtain structures of the peptides and releasing factors. Provide basic information for structure/activity studies leading to mimetics. Develop receptor assays suitable for screening mimetics.

2. **Significance**: Water and ion balance are critical factors for the well being of insect. Rapid development of mimetics that disrupt those factors could be of great commercial value while being environmentally sound.

3. **Constraints**: None

Current and Future Cooperators

* G. M. Coast, Univ. of London, London, U.K.
* L. Schoofs, Katholiecke University, Leuven, Belgium
* D. R. Nässel, Univ. of Stockholm, Stockholm, Sweden
* N. DeDecker, Limburgs University, Diepenbeek, Belgium
* T. Pannabecker, Cornell University, Ithaca, NY
* K. L. Beyenbach, Cornell University, Ithaca, NY
* T. K. Hayes, Texas A&M University, College Station, TX
* S. M. Meola, FAPRL-ARS-USDA, College Station, TX
* R. J. Nachman, FAPRL-ARS-USDA, College Station, TX
* D. L. Bull, FAPRL-ARS-USDA, College Station, TX

Potential Uses of Research Findings

Analog studies leading to stable but toxic peptide mimetic compounds suitable for commercial sales.

Thoughts on Research Needs

Must be aware of any technological breakthroughs and be prepared to exploit immediately.

Areas of Insect Neurobiology Research Which are Not Being Addressed Within ARS, but Have Potential for Breakthroughs

* Neuropeptide releasing factors
* Naturally-occurring plant toxins

Insect Pest Species That Might Be Vulnerable to a Neurobiology Approach but at Present are Not Being Studied With That Approach in Mind

Small, but important insects and arthropods. Mosquitos, aphids, ticks, mites.

Name: **Eric Jang**
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Research Accomplishments

1. Established primary cell cultures from three species of tephritid fruit flies. Identified heat-shock proteins and established ecdysteroid sensitivity in cells.

2. Determined electrophysiological responses of tephritid fruit flies to host fruit odors, plant volatiles, attractants, pheromones and other behaviorally relevant semiochemicals. Established behavioral responses of fruit flies to pheromones and host fruit odors.

3. Established the importance of accessory glands in modulation of pheromone /host fruit odor driven behavior in female medflies.

4. Identified physiological effects of benzodioxoles on in vitro biosyntheses and release of JH from medfly corpora allata

Research Objectives

1. **Purpose**: Identify physiological and biochemical factors which influence fruit fly and parasite chemoreception and behavior. Identify physiological and biochemical factors which regulate fruit fly and parasite growth and development. Establish structure/activity relationships of fruit fly chemoreceptors to semiochemicals.

2. **Significance**: The physiological and biochemical basis of fruit fly and parasite chemoreception, behavior, growth and development is not well understood. Knowledge of how insect hormones, neurohormones, neuropeptides and other related compounds function in fruit flies may lead to improved methods for monitoring, detection, mass-rearing or control of these economically important pest species.

3. **Constraints**: The paucity of basic information on fruit fly physiology/ biochemistry requires that this information be gathered. The extremely small size of the antennal receptors require the development of suitable techniques to record from these receptors. Time and manpower will be needed to complete these objectives.

Current and Future Cooperators

* Doug Light, Bob Flath, Bruce Campbell, WRRC, Albany, CA
* Barbara Leonhardt, James Avery, Al DeMilo, ICEL, Beltsville
* Dick Dickens, BWRU, Behavioral Physiology, Mississippi State
* Other university, state and federal researchers

Solving High Priority Pest Problems

Exotic pest introductions continue to threaten U.S. agriculture. Improved attractants for use in population monitoring, control and eradication will aid in preventing the introduction of fruit flies into the U.S. Improved mass-rearing of fruit flies will improve the competitiveness of sterile flies used in control programs. Additional information on the physiology and biochemistry of fruit flies will expand our knowledge of potential candidate control strategies which may be useful in development of new techniques. This information will be extremely useful to federal action agencies such as APHIS as well as state regulatory groups responsible for exotic pest exclusion activities.

Thoughts on Research Needs

Basic studies on fruit fly neurobiology are needed to expand our knowledge of these insect pests. Close interaction with researchers working on other diptera may be helpful in defining potential target systems which may be useful in ongoing and future research programs.

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CRIS #: 1275-22000-069-OOD (50%), 1275-22000-070-OOD (50%)
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Research Accomplishments

In cooperation with other INHL scientists:

1. Developed in vitro and in vivo bioassays for gypsy moth prothoracicotropic hormone (PTTH).

2. Characterized gypsy moth vitellogenins and vitellin and demonstrated a novel suppression mechanism by juvenile hormone.

3. Demonstrated ecdysone and 3-dehydroecdysone production by gypsy moth prothoracic glands and a novel egg ecdysone ketoreductase.

4. Demonstrated a novel form of PTTH in gypsy moth eggs.

5. Obtained a partial nucleotide sequence for the gypsy moth PTTH gene.

Research Objectives

* (CRIS #1275-22000-069-OOD) Identify physiological processes by which the insect central nervous system controls and regulates development, reproduction and diapause, and develop bioassays for these factors.

* (CRIS #1275-22000-070-OOD) Identify, isolate, and structurally characterize neurohormones and related peptidergic factors from the insects.

1. **Purpose**: To develop knowledge of hormonal and neurohormonal regulation of insect growth, development, and reproductive processes: specifically peptide, gene and receptor isolation for mosquito *(Aedes aegypti)* EDNH and gypsy moth *(Lymantria dispar)* PTTH.

2. **Significance**: This research will provide a basis for discovering and developing new insect control principles.

3. **Constraints**: Inadequate knowledge of these factors and their regulatory mechanisms hampers the development of new control methods.

Current and Future Cooperators

* Terry Adams, USDA, ARS, BRL, Fargo, North Dakota
* Andrew Chen, USDA, ARS, FAPRL, College Station, Texas
* Bruce Black, American Cyanamid Company, Princeton, New Jersey
* Lois Miller, University of Georgia, Athens
* David Borst, Illinois State University, Normal
* Howard Fescemyer, Clemson University, Clemson, South Carolina
* M. Aruchami, Kongunadu Arts and Science College, Coimbatore, India
* INHL Scientists

Potential Uses of Research Findings

This research is expected to develop the knowledge and tools necessary for examining use in insect control by direct expression of the natural products (i.e. neuropeptide, receptor) in insect vectors. Alternatively, it will provide the knowledge necessary for developing potent analogs to these neuropeptides which can be expected to block insect growth and/or reproduction. It is expected that products will eventually be developed for insect control in the form of biological vectors (i.e., engineering baculoviruses, etc.) or as peptidomimetics.

Thoughts on Research Needs

The molecular biology of PTTH and other insect ecdysiotropins is currently being addressed in ARS by temporary employees and needs permanent funding of a molecular biologist position. Full sequences are nearly available for mosquito *(Aedes aegypti)* EDNH, gypsy moth PTTH and testis ecdysiotropin. The information in these sequences can best be exploited by a permanent molecular biologist who would isolate genes and develop vectors for expressing inhibitory analogs. Such vectors would be expected to block insect development and potentially be useful in insect control and IPM programs.

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Research Accomplishments

1. Synthesized 3 cecropin analogs (41-43 residues each)
2. Synthesized series of analogs and derivatives of PBAN for ongoing research
3. Synthesized series of peptides from flies and several pseudopeptide analogs
4. Developed new synthesis of a tertiary alcohol proline linker for use in c-terminal proline peptide synthesis.

Research Objectives

Continue work on synthesis of peptides, analogs, and peptide-mimetics. Continue work on new peptide linkers.

1. **Purpose**: Elucidating insect neurohormone metabolism and developing improved synthetic methods.

2. **Significance**: Eventually applicable to development of potential insect controls, but primarily for study of insect development. New linkers would be broadly applicable to all areas of peptide synthesis.

3. **Constraints**: Time it takes procurement to order supplies/equipment.

Current and Future Cooperators

* Renee Wagner, USDA, ARS, Livestock Insects Lab, Beltsville, MD
* Mark Holman, USDA, ARS, FAPRL, College Station, TX
* Lowell Owens, USDA, ARS, Plant Mol. Biol. Lab., Beltsville, MD
* Mike Bausher, USDA, ARS, Horticultural Research Lab., Orlando, FL
* Berne Jones, USDA, ARS, Barley & Malt Lab., Madison, WI
* Eliot Herman, USDA, ARS, Plant Mol. Biol. Lab., Beltsville, MD
* Thomas H. Wise, USDA, ARS, Reproduction Research, Clay Center, NB
* Thomas Bach, Universitat Karlsruhe
* Dave Carlson, USDA, ARS, Genetics & Mol. Biol. Research, Gainesville, FL

Potential Uses of Research Findings

Peptide-encoding genes can in theory be inserted into viruses allowing "inappropriate" hormonal stimulation of target insects. Peptide-mimetics may be prepared that could be absorbed through insect cuticle, but would have to be extremely powerful or extremely cheap to compete with conventional pesticides. Packaging would be the same as for regular viruses used to control insects, or conventional pesticides, respectively.

Thoughts on Research Needs

Speeding up the procurement process would definitely help.

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Research Accomplishments

The study of antennal chemoreceptivity has led to behavioral resolution of:

1. the attractive components of the complex, pheromonal odor of calling male Medflies;

2. the synergism of green leaf volatiles and the Medfly pheromone;

3. the response of Medflies to fruit odors being enhanced by certain fruit volatiles and interrupted/inhibited by others;

4. the enhancement/synergism of lepidopteran pheromones by green leaf volatiles and other host plant volatiles.

Research Objectives

Olfactory Neurobiology: Find new semiochemicals (attractants, disruptants/inhibitors, etc.) through study of the chemoreceptive processes of excitation and inhibition that dictate the subcellular, cellular, and network processes of sensory transduction, conduction, and integration.

* Study the antennal and receptor sensitivity and selectivity of fruit flies and moths *(H. zea)* for host plant blossom-, fruit-, and leaf-odors and conspecific pheromones.
* Study the integrative processes of the CNS for discrimination and selective sensitivity for complex odors; pheromonal, host-plant kairomonal, and both of these in their natural concert.

1. **Purpose**: To resolve the selective receptivity to plant and conspecific volatiles, demonstrate their semiochemical activities, and then develop them into a new class of effective attractants or disruptants for insect monitoring and control strategies.

2. **Significance**: Effective attractants for female insects are lacking, due in part to our inability to successfully study natural semiochemical odors that are compositionally complex. Neurophysiology/ethology offers a means to identify both sensitivities to individual constituents and the sensory mechanisms of synergism and interactions between these components in a complex odor(s).

3. **Constraints**: A Time/Manpower Commitment: Both peripheral receptor neurons and the CNS interneurons are minute and numerous, thus requiring considerable time to conduct thorough recordings and samplings of the neural elements that will allow for an adequate comprehension, modeling, and then manipulation of these insect olfactory systems.

Current and Future Cooperators

* Bob Flath, Roy Teranishi, Saima Kint, Ron Buttery, Ron Binder, USDA-ARS-WRRC, Albany, CA;
* Eric B. Jang, USDA-ARS, Hilo, HI;
* Dick Dickens, USDA-ARS, Mississippi State, MS
* Tom Christensen, Mark Willis & John Hildebrand Arizona Research Lab., Div. of Neurobiology University of Arizona, Tucson, AZ

Solving High Priority Pest Problems

1. Aid and guide in the identification and resolution of semiochemicals and novel monitoring and control strategies that are almost entirely lacking for female insects;

2. Discover and develop appropriate and powerful behavioral modulators, both synergists and inhibitors, based on knowledge of their neuronal mode of actions at both the level of chemoreception and perception.

Thoughts on Research Needs

The Integration of more of ARS' research skills through Cooperation; i.e. Once semiochemicals are identified and the olfactory receptive and perceptive systems are being analyzed, then the expansion of experiments and cooperation to include the effects and influences of neurohormones, -peptides, -amines, and other neuro-modulators on these model olfactory systems would be of great timeliness and benefit for integration of many aspects of ARS' commitment to insect neurobiology. Also needed is analysis and resolution of: (1) Olfactory receptor molecules (proteins), (2) Receptor-site Transduction, (3) CNS Network and Multifiber Integration Systems (i. e., normal function of simple brains), (4) Multimodal (various sensory systems: vision, mechanoreception, thermal, olfaction, etc.) Integration. (5) Adaptation and Habituation systems, (6) Mechanisms of Learning/Adaptation.

Name: **Marcia J. Loeb**
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Research Accomplishments

1. Testis ecdysiotropin (TE), a neuropeptide from brains of *Heliothis virescens* and *Lymantria dispar*, stimulates ecdysteroid synthesis by testis sheaths of either species.

2. TE from *L. dispar* has been partially purified and characterized; several molecular weight species exist.

3. At the cellular level, the TE-testis system requires Ca^{2+} titers and second messengers different from those operative in the PTTH-prothoracic gland system. It is a separate ecdysiotropic system.

4. Exposure to ecdysteroids induces testis sheaths and fat body tissue to secrete peptidic/lipoproteinaceous growth factors. The growth factors induce development of male reproductive tracts during pupal stages of H. virescens and L. dispar.

5. At least nine-molecular weight species of growth factors are released by testis sheaths and fat body exposed to ecdysteroids.

Research Objectives

Identify sequences for all forms of TE, and determine bioactive groups. Synthetic analogs may be more or less active, or block the action of TE by sticking to TE receptors. Prepare antibodies against a moiety containing active groups; do histochemical studies to reveal the source and means of dispensing TE in vivo. Find the gene(s) for the families of TE, and determine the relationships between the different forms. Study the mechanisms of genetic and physiological control over TE synthesis and release. Isolate and identify sequences for one or more reproductive tract growth factors, and determine modes of action and interaction at the cellular level. Probe a cDNA library of active tissues with known mammalian growth factor sequences and/or insect growth factor antibodies to determine genes.

1. **Purpose**: To elucidate the hormonal and neurohormonal mechanisms controlling maturation of the male lepidopteran reproductive tract.

2. **Significance**: Lepidopteran pests are prevalent worldwide. During the pupal stage, male reproductive tracts change from rudimentary to complicated structures ready to deliver mature sperm to receptive females. If the regulation of male reproductive tract development is understood, it may be possible to develop antagonists to one or more of the controlling factors and thus block male sexual development. If feasible in the field, these materials could serve as male contraceptives.

3. **Constraints**: The physiology of the male insect reproduction involves a complicated cascade of events. Untangling the web of cross-interactions requires intensive research and personnel at technical and post-doctoral levels.

Current and Future Cooperators

* Robert Bell, INHL Beltsville, MD
* Dale Gelman, INHL Beltsville, MD
* Jan Kochansky, INHL Beltsville, MD
* Bill Lusby, INHL Beltsville, MD
* Dwight Lynn, IBL, Beltsville, MD
* Renee Wagner, LIL Beltsville, MD
* S. Meola, FAPRL, USDA College Station TX
* J. Wright, Texas A & M University, College Station, TX
* R. Davis, INHL, Beltsville, MD

Potential Uses of Research Findings

It is desirable to block the reproductive activity of lepidopteran pests. Determination of neuropeptides and other peptide and non-peptidic factors necessary for the development of reproductively-competent males will provide a better understanding of male reproductive physiology. Identification of key factors may lead to synthesis of altered mimics which may block receptors for active peptides or enzymes in the cascade which will block activity. It is hoped that future mimics or analogs may lead to environmentally safe methods of interrupting the reproductive process at a vulnerable stage.

Thoughts on Research Needs

Although equipment and supplies are available, additional personnel to use them effectively is lacking. It would help my program if I could hire a full time technician and a post doctoral associate to address the surfeit of research problems.

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Research Accomplishments

1. Identified and patented cholesterol oleate as the mounting sex pheromone for several species of hard ticks including *Dermacentor variabilis, D. andersoni,* and *Amblyomma americanum.*

2. Elucidated the structure of natural product which is lethal to greenhouse whitefly (Trialeurodes vaporariorum), sweetpotato whitefly (*Bemisia tabaci*), and other pests of economic significance.

3. Isolated and identified compounds from setal exudate of *Corythucha cydoniae* and *C. ciliata* which possess antimicrobial properties.

4. Developed a battery of gas-phase mass spectrometric methods for probing molecular structure of carotenoid materials at 100 times the sensitivity of prior methods.

5. Developed mass spectrometric method for the confirmation of the herbicide Tebuthiuron (Spike) at the 500 femtogram level.

Research Objectives

To elucidate or confirm the molecular structure of biologically active natural and synthetic products.

1. **Purpose**: To identify or confirm the structure of neuropeptides, ecdysteroids, analogs, and mimics.

2. **Significance**: Make possible design of bio-rational control measures for the control of economically significant insects.

3. **Constraints**: Additional bench space needed.
Additional technician needed.

Current and Future Cooperators

* Mark Feldlaufer, ARS, PSI, INHL, Beltsville, MD
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* Gunter Weirich, ARS, PSI, INHL, Beltsville, MD
* Dale Gelman, ARS, PSI, INHL, Beltsville, MD
* Thomas Kelly, ARS, PSI, INHL, Beltsville, MD
* Marcia Loeb, ARS, PSI, INHL, Beltsville, MD
* Peter Masler, ARS, PSI, INHL, Beltsville, MD
* Ashok Raina, ARS, PSI, INHL, Beltsville, MD
* James Tumlinson, ARS, IABBBL, Gainesville, FL
* Peter Teal, ARS, IABBBL, Gainesville, FL
* Renee Wagner, ARS, PSI, LIL, Beltsville, MD

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Research Accomplishments

1. Discovered and characterized embryonic and post-embryonic *Lymantria dispar* PTTH, identified two molecular weight classes of this ecdysiotropin and outlined this bi-molecular distribution in other lepidopterans.

2. Discovered a non-cerebral neuro haemal center in *L. dispar* which functions independent of the brain to control development and metamorphosis, have partially characterized an ecdysiotropic peptide (APET) from this center, and have developed a bioassay for the peptide.

3. Isolated, purified and sequenced a pheromonotropic peptide (PBAN) from adult brains of *L. dispar*, the first neuropeptide ever sequenced from gypsy moth.

4. Discovered and characterized neuropeptide-metabolizing, membrane-bound enzyme activities in brain preparations; endopeptidase and aminopeptidase activities were described.

5. Purified, determined amino acid composition and obtained a partial amino acid sequence for a mosquito EDNH; identified multiple molecular forms of EDNH and identified a synergistic effect of these forms in vivo.

Research Objectives

We intend to completely sequence EDNH, isolate and characterize PTTH and APET, conduct structure-function studies on EDNH and gypsy moth PBAN, isolate the PBAN, EDNH and PTTH genes, and characterize specific neuropeptide metabolizing enzymes.

1. **Purpose**: All studies are directed toward understanding the production, action, and degradation of specific insect neuropeptides which are essential f or reproduction and development.

2. **Significance**: The studies will provide a comprehensive, vertical knowledge of neuropeptide systems which is necessary for the development of insect control agents through peptidomimetic design, baculovirus engineering, and other technologies.

3. **Constraints**: Essential for rapid progress in these cutting-edge programs are molecular biologists, and support scientists trained in protein and enzyme manipulation.

Current and Future Cooperators

* T.S. Adams (ARS, Fargo)
* R.B. Imberski (U. of Maryland)
* T.A. Coudron (ARS, Columbia)
* J.W. Gerst (N. Dakota State Univ.)
* B. Black (American Cyanamid)
* R. Wagner (ARS, Beltsville)
* D. Bolt (ARS, Beltsville)
* Members of INHL

Potential Uses of Research Findings

Research findings will be of value as 1) direct avenues to the development of experimental, novel pest control agents ; and 2) as highly detailed reservoirs of information and methods relative to neuropeptide production, action and consequence in insect and other pest species. This will significantly decrease the time and research expense required for the identification of neuropeptides which control key physiological events and for the development of agents which will interfere with these events.

Thoughts on Research Needs

ARS must continue to lead in the area of neuropeptide metabolism and action. This is especially critical with studies on economically important insects, absent from the focus of non-ARS programs. The return on modest investments in trained personnel and new technology in peptide chemistry and molecular genetics will be significant.

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Research Accomplishments

1. Measured sensitivity and selectivity of three *T. ni* antennal pheromone specialist receptor neurons to six female sex pheromone components at natural and elevated stimulus intensities. Demonstrated that specialist neurons respond to only one of the pheromone components at naturally relevant stimulus intensities. However, when stimulus intensities become excessive, the neurons will respond to all of the other pheromone components,

2. Developed an assay of discrimination for sex pheromones, demonstrating that *T. ni* discriminates differences between concentrations of Z7-12:Ac, excised sex pheromone glands and Z7-12:Ac, *inter alia*,

3. Determined that *T. ni* males discriminate a difference between a 3-component mixture and a 6-component pheromone mixture, providing evidence that at least four antennal specialist neurons may exist.

4. Conducted ancillary electrophysiological experiments of individual specialist neurons of *T. ni* and *H. zea* with various pheromone congeners and analogs.

5. Conducted specialized assays of *T. ni* discrimination to determine the effect of two 14-carbon acetates on the appetitive flight response and determine how these two components modify and contribute to discrimination.

Research Objectives

To understand brain functions that regulate and control pheromone based appetitive upwind flight and discrimination. The approach is to measure the sensory input in the form of antennal specialist neuronal responses and infer brain function from discriminative and appetitive behaviors. To this end, human psychological and psychophysical approaches are applied. Alternative pheromone analogs and congeners will be investigated to determine their effect on both the peripheral sensory system and their effect an behavior.

1. **Purpose**: To provide new approaches to and a deeper understanding of pheromone-elicited behaviors. To provide new measures of neuroagonist and antagonist effects.

2. **Significance**: The ability to more completely confuse and inhibit pheromone-elicited behavior to preclude mating has significant importance to future biorational field control measures. Signal achievements in the area of field control have been slowly forthcoming but a deeper understanding of sensory input/behavior output is necessary for the future. Some of the approaches suggested provide new platforms from which to measure the effects of neuroagonists.

3. **Constraints**: The electrophysiological and behavioral studies outlined above require particular care in the determination and reproduction of relevant stimulus intensities for assay. The assays of discrimination are new and the assessment of mixture effects requires significant replication, particularly at low stimulus intensities. Electrophysiological studies are usually difficult.

Current and Future Cooperators

* R.J. O'Connell, Worcester Fndtn.
* A.J. Grant, Worcester Fndtn.
* J.C. Dickens, USDA
* C.D. Prestwich, SUNY
* R.E. Doolittle, USDA
* J.H. Tumlinson, USDA
* J.R. McLaughlin, USDA
* R.K Saini, ICIPE
* E.T. Chao, Berea College
* C.O. Caulkins, USDA

Potential Uses of Research Findings

An insect must discriminate its particular congeneric sexual signal from similar heterogeneric signals. From wind tunnel experiments in progress, it appears that slightly modified pheromone mixtures will completely disrupt discrimination and upwind flight. These modified mixtures are effective at natural release rates. If further research confirms the preliminary studies, biologically requisite behaviors may be more easily prevented or reduced than by using inundative semiochemical releases. Even if mating is not prevented entirely, it follows that an insect whose ability to discriminate is disrupted is less likely to find a mate. Mixture effects on the ability to discriminate and the ensuing effect on mating in the field environment have to be evaluated.

Synthetic super agonist and antagonist molecules will be evaluated by electrophysiological and behavioral assays. Such molecules could be used to more effectively disrupt field behaviors than inundative releases of congeneric semiochemical components.

Thoughts on Research Needs

Specialized studies of pheromone congeners and analogs on antennal specialist neurons may lead to new inhibitors of neuronal function and/or provide probes for investigating receptor function. Investigations of single specialist receptor cell responses are the only way to determine whether or not and how such compounds affect one or more particular specialist neurons. In theory, if a single specialist receptor neuron such as the HS(a) of *T. ni* that detects Z7-12:Ac could be inactivated, it is likely that little to no pheromone-directed flight could take place in the field.

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Research Accomplishments

1. Localization of leucomyosuppressin (the first inhibitory peptide isolated from an insect) in the cockroach *Leucophaea maderae* and the stable fly *Stomoxys calcitrans* using immunocytochemistry.

2. Discovery of the pericardial neurohemal in cyclorraphid flies and that it is present only in the adult stage. This neurohemal organ is larger than the corpus cardiacum, previously the largest release/storage site in these insects.

3. Site of a new secretory center in the synganglion of the adult lone star in cooperation with Dr. J. M. Pound of the Kerrville, USDA laboratory, Tick Research Unit.

Research Objectives

Determination of peripheral neurosecretory systems in pest species of arthropods.

Localization of sites of synthesis, release, and receptors of various insect neuropeptides.

1. **Purpose**: To form a basis for understanding the neurohormone system of arthropods. This system influences all activities of these animals either directly or indirectly. This knowledge will be used to develop a means of controlling these insects via interruption of this system.

2. **Significance**: The neuroendocrine system of arthropods not only acts directly as a regulatory system of various physiological activities, but also as an intermediary system between the endocrine and nervous system, and thus disruption of this system can have both short and long-term effects on these animals.

3. **Constraints**: The major constraint at this time is determination of peptide analogs that will affect only specific pest species and the delivery of these compound to target sites in the insects.

Current and Future Cooperators

Dr. Marcia Loeb, USDA, ARS, Beltsville, MD
Dr. Dale Gelman, USDA, ARS, Beltsville, MD
Dr. J. M. Pound, USDA, ARS, Kerrville, TX

Name: **Ronald J. Nachman**
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Research Accomplishments

1. Structure-activity studies on sulfakinin, leucokinin, pyrokinin, and myosuppressin insect neuropeptide families; including discovery of active core, critical amino acids, superagonist analogs, and an antagonist analog.

2. First active pseudopeptide analog of an insect neuropeptide. Also, a stable replacement for labile post-translational modification was incorporated into an insect neuropeptide analog.

3. First bifunctional, heterodimeric analog of an insect neuropeptide.

4. First characterization of active conformation of an insect neuropeptide family (pyrokinins or PBAN-like family).

5. Characterization of active conformation of the achetakinin/leucokinin insect neuropeptide family.

Research Objectives

1. Structure-activity and active conformation studies of established and newly-discovered insect peptide families.

2. Synthesize and evaluate pseudopeptide modifications of established and new insect neuropeptides.

3. Develop antibodies to active, restricted conformation analogs as receptor models.

4. Utilize synthetic turn-mimetic systems to prepare neuropeptide mimics.

5. Non-peptide mimetic development.

Current and Future Cooperators

* G.M. Holman, FAPRL/ARS, College Station, TX
* R. Beier, FAPRL/ARS, College Station, TX
* F. Clottens, FAPRL/ARS, College Station, TX
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* M. Horiharan, Scripps Research Institute, La Jolla, CA
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* M. Khan, Univ. of Washington, Seattle, WA
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* Dr. D. Konopinska, Wroclaw Univ., Wroclaw, Poland

Potential Uses of Research Findings

The research goal is to develop pseudopeptide and non-peptide agonists/antagonists capable of disrupting the internal balance of insects that are maintained by neuropeptides. Such analogs are potential candidates for selective, environmentally-safe insect pest management agents of the future. Such agents may be suitable for delivery by traditional methods.

Thoughts on Research Needs

Receptor isolation for direct analog binding studies; improved bioassay systems; mimetic agonists/antagonists; and eventually, delivery systems for mimetics.

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Research Accomplishments

1. Regulation of Pheromone Biosynthesis:

* Target site of PBAN was elucidated (Teal, Tumlinson, Oberlander)
* Pheromone production was suppressed by a substance from various tissues of virgin female moths (Teal) Tumlinson, Oberlander)

2. Tissue culture of Moth Brains:

Primary cultures of neuronal and glial cells were optimized for survival and the production of active materials (Oberlander, Oland, Hildebrand)

Research Objectives

Develop improved tissue culture systems for studying the development of the insect nervous system.

1. **Purpose**: Develop improved primary cultures of cells from the insect nervous systems and attempt to establish a cell line(s) from such cells.

2. **Significance**: Developmental and other regulatory molecules in the central nervous system can be discovered, identified and investigated with the aid of tissue culture methodology.

3. **Constraints**: The tissue culture methodology for the insect nervous system currently available is primitive by comparison with the understanding that we have of vertebrate neurons and glial cells in culture. There are no cell lines from the insect nervous system; and glial cells die quickly in primary culture. These technical constraints must be overcome.

Current and Future Collaborators

* James Tumlinson (ARS)
* Peter Teal (ARS)
* John Hildebrand (University of Arizona)
* Lynne Oland (University of Arizona)

Solving High Priority Pest Problems

This research to long-term basic research aimed at understanding post-embryonic development of the insect nervous system, and especially the olfactory system. It is expected that this research approach will lead to the discovery of new regulatory molecules that may be manipulated for pest control.

Thoughts on Research Needs

Neurobiological research is at the forefront of medical research, but is seriously lacking in entomology. It is time to take advantage of the significant advances being made in this area and apply them to insects.

Areas of Insect Neurobiology Which are Not Being Addressed Within ARS but Have Potential for Breakthroughs

The area of developmental neurobiology is not being addressed by ARS, and the proposed research is an effort in that direction.

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Research Accomplishments

1. Isolated, identified and demonstrated biological activity of 4 peptides of AKH family from a moth and a fly. Established a system of nomenclature for insect peptides.

2. Discovered, isolated and identified PBAN from H. zea. Cloned and sequenced the gene for PBAN. obtained polyclonal antibodies and did immunohistochemistry. Developed PBAN specific ELISA and RIA. Cloned PBAN gene in baculovirus and obtained in vitro expression.

3. Discovered that pheromone production in wild corn earworm females is regulated by plant volatiles, including the plant hormone ethylene.

4. Discovered, isolated and identified a pheromonostatic peptide (PSP) from the accessory glands of male corn earworm. PSP is transferred to the female at the time of mating to terminate pheromone production in mated females.

5. Conducted structure activity studies on PBAN.

Research Objectives

* Design analogs and mimics of PBAN.
* Isolate and identify the receptor for PBAN.
* Synthesize PSP and elucidate its mode of action.
* Conduct structure activity studies on PSP to identify smallest biologically active sequence.
* Clone the gene for a PSP analog into a suitable vector, that will not effect the survival of the insect but express the peptide.
* Isolate and identify the diuretic and anti-diuretic factors in H. zea.

1. **Purpose**: To understand the behavioral physiology of reproduction in H. zea and other important species of moths. To exploit weak links in these processes that could be used to disrupt reproduction.

2. **Significance**: Technology obtained through this research could be used to develop environmentally safe pest management practices.

3. **Constraints**: (1) Need a permanent insect molecular biologist. (2) Need a vector for expression of a desired peptide in insects. The vector itself should not be lethal to the host insect.

Current and Future Cooperators

* J. P. Kochansky, ARS, Beltsville
* E. P. Masler, ARS, Beltsville
* C. F. Weirich, ARS, Beltsville
* R. Wagner, ARS, Beltsville
* W. Lusby, ARS, Beltsville
* J. M. Giebultowicz, Visiting Scientist, Beltsville
* V. Vakharia, Center Ag. Biotech. Univ. MD, College Park
* G. D. Prestwich, SUNY, Stony Brook, New York
* A. Rafaeli, Univ. Tel Aviv, Israel
* W. Roelofs, Cornell Univ., Geneva, New York
* D. Bushman, Mount Saint Marys College, Maryland

Potential Uses of Research Findings

Once we identify a peptide antagonist to PBAN or a peptide or nonpeptide mimic to PSP, we can either develop linkage with a commercial company to determine mechanisms by which such molecules can be delivered to the target insect. We can on our own develop a vector system to deliver the desired peptide, and then transfer the technology to a company.

Thoughts on Research Needs

Need to set up various bioassays for different peptides. We need an in-house facility to do amino acid analysis. A permanent molecular biologist is top priority.

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Research Accomplishments

1. Determined the metabolic pathways of ecdysteroids during pupal-adult development (Ecdysteroid acids and 3-epiecdysteroids are major metabolites) and determined the profile of ecdysteroids during embryonic development (embryos are not capable of ecdysteroid biosynthesis, but can enzymatically convert maternally-derived ecdysteroids).

2. Investigated sterol metabolism in Diptera (housefly and Drosophila) related to ecdysteroid biosynthesis.

3. Identified new intermediates of phytosterol metabolism in *Spodoptera litteralis* and studied effects of sterol metabolism inhibitor.

Research Objectives

Study feasibility of genetically altering sterol composition in certain crop plants and effects on development of crop pest insects.

1. **Purpose**: To disrupt pest insect development by depriving them of sufficient utilizable dietary sterols.

2. **Significance**: To provide environmentally-sound strategies to control insects based on exploiting unique aspects of insect biochemistry.

3. **Constraints**: Lack of permanent SY with expertise in molecular biology; lack of sufficient technical support.

Current and Future Cooperators

* R. Imberski, University of Maryland, College Park, MD
* E. Bernays, University of Arizona, Tucson, AZ
* W.D. Nes, ARS, USDA, Russell Research Center, Athens, GA
* H.H. Rees, University of Liverpool, U.K.

Potential Uses of Research Findings

Interference with sterol utilization or molting hormone biosynthesis would form the basis of a control strategy based on a unique difference between insect and mammalian biochemistry.

Thoughts on Research Needs

Analytical instrumentation is currently state-of-the-art. It will be necessary to maintain this strong analytical capability to unequivocally identify bioactive compounds and their intermediates.

Name: **Peter E. A. Teal**
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CRIS #: 6615-22000-OOD
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Research Accomplishments

1. Determined that the abdominal nerve cord is involved in regulation of sex pheromone production in *Heliothis* moths.

2. Discovered a hormone produced by senescing virgin *Helicoverpa zea* females that inhibits the action of pheromone biosynthesis activating neuropeptide.

3. Discovered that the terminal abdominal ganglion of *Heliothis* moths innovates the pheromone gland and that the octopamine will stimulate sex pheromone production.

4. Isolated three pheromonotropic peptides from the brain and terminal abdominal ganglion of *Heliothis virescens*.

Research Objectives

* Elucidation of neural factors regulating the inhibition of pheromone production in moths.

* Identification of pheromonotropic neuropeptides from brains and other neural centers in moths.

* Elucidation of neural factors regulating stimulation and inhibition of pheromone production in fruit flies.

1. **Purpose**: All objectives are aimed at the isolation identification and determination of the modes of action of neural factors that regulate the production and termination of sex pheromone production in pest insects.

2. **Significance**: Sex pheromones are required for reproduction in insects and the mechanisms that regulate induction and termination of pheromone biosynthesis are key components of the communication system. Therefore, development of methods to inhibit or alter biosynthesis of sex pheromones based on neurally produced compounds may provide highly effective alternatives to classical pesticide control.

3. **Constraints**: More resources and personnel are required to accomplish research goals in a reasonable period of time. Because of its complexity this research is time consuming and many different skills are required to conduct experiments. A post doctoral associate would aid considerably in achieving research objectives.

Current and Future Cooperators

* J.H. Tumlinson, IABBBRL, USDA, ARS, Gainesville, Florida
* H. Oberlander, IABBBRL, USDA. ARS, Gainesville, Florida
* T. Christensen, Division of Neurobiology, University of Arizona, Tucson, Arizona
* J. G. Hildebrand, Division of Neurobiology, University of Arizona, Tucson, Arizona

Potential Uses of Research Findings

One direct and immediate use of results that elucidate insect pheromone biosynthesis is the identification of more complete and useful pheromones for important pests. Pheromones have already been demonstrated to be effective for both monitoring and control of many species but complete pheromone blends are not yet available for several important pests.

Research Needs

The major need for continued success in these areas is the availability of in house analytical instrumentation required for identification of natural products. At present peptide sequence analysis, amino acid analysis and FAB mass spectrometry are not available for routine studies in the agency. Therefore, we are required to expend significant resources for these analyses at other centers. One way to overcome this would be to establish a center within the agency that would conduct these studies at nominal cost and on a routine basis for ARS scientists.

Name: **James H. Tumlinson**
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Research Accomplishments

1. Host detection by semiochemically mediated associative learning in parasitic wasps.

2. Neural regulation of sex pheromone biosynthesis in *Heliothis* moths.

3. Identification of *Manduca sexta* sex pheromone.

4. Exploitation of herbivore-induced plant odors by host-seeking, parasitic wasps, and systemic release of chemical signals by corn.

5. Endogenous suppression of pheromone production in virgin female moths.

Research Objectives

1. Elucidate the pheromone biosynthetic pathway in key moth and fruit fly pests and the factors, including neuropeptides, that regulate pheromone biosynthesis in these insects.

2. Identify the semiochemicals and behavioral mechanisms that regulate host foraging in key parasitoid species of important pest insects.

3. Determine the identity and signal transduction mechanisms that trigger the release of plant volatiles that act as signals for beneficial insects including parasitoids.

4. Identify complete pheromone blends for important pests for which useful pheromones are not yet available.

Current and Future Cooperators

* W.J. Lewis, Insect Biology/Management Systems Research, USDA, ARS, SAA, IBPMR, Georgia Coastal Plains Experiment Station, P. 0. Box 748, Tifton, Georgia 31793
* John Law, University of Arizona, Biotechnology Center. Biological Science West 364 Tucson, Arizona 85721
* John Hildebrand, University of Arizona, Arizona Research Laboratories, Division of Neurobiology, 603 Gould-Simpson Science Bldg., Tucson, Arizona
* Tom Christensen, University of Arizona, Arizona Research Laboratories, Division of Neurobiology, 603 Gould-Simpson Science Bldg., Tucson, Arizona 85721
* C.A. Ryan, Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164
* L.E.M. Vet, Agriculture University of Wageningen, Department of Entomology, P.O. Box 8031, 6700 EH Wageningen THE NETHERLANDS
* Miklos Toth, Hungarian Academy of Sciences, Plant Protection Institute, H-1525 Budapest, P.0.B. 102 HUNGARY

Potential Uses of Research Findings

One direct and immediate use of results that elucidate insect pheromone biosynthesis is the identification of more complete and useful pheromones for important pests. Pheromones have already been demonstrated to be effective for both monitoring and control of many species but complete pheromone blends are not yet available for several important pests.

Semiochemicals that mediate the foraging behavior of beneficial entomophagous insects will be extremely useful in retaining beneficial insects in a target area and enhancing their effectiveness. It is possible that plants may be bred or engineered to be more attractive, to beneficial insects in the future.

Research Needs

ARS resources are spread too thinly to be effective in most areas. Resources need to be concentrated in research groups that are productive and have demonstrated excellence in a particular area in order to achieve the critical mass, necessary to achieve important objectives.

Name: **Renee M. Wagner**
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CRIS #: 1265-32000-033-OOD
Phone: (301) 504-9168
FAX #: (301) 504-9062

Research Accomplishments

1. Isolation & characterization of receptor for AKH in face fly.

2. Design & testing of metabolically-stable analogs which block receptor in face fly.

3. Isolation & characterization of new reproductive peptides from male & female house fly.

4. Isolation & characterization of new reproductive peptides from male & female stable fly.

5. Determination of metabolic pathway for metabolism of AKH in face fly.

Research Objectives

* Characterization of additional reproductive peptides from face, house, stable fly.
* Design of additional analogs for testing in face, house, stable fly.
* Design of compounds for testing in the field on house, face, stable fly based on analog studies
* Utilize molecular biological approaches for control of flies based on peptide information.
* Isolate receptor(s) for most promising of reproductive peptides & determine mode of action

1. **Purpose**: Control of insect pests of livestock & poultry. Provide information to industry for development of "rational" means of insect pest control.

2. **Significance**: Part of integrated pest management research at Beltsville. Provide basis for possible funding by industry for applied studies.

3. **Constraints:** Lack of technical support. Need interface with applied work: formulations chemist?

Current and Future Cooperators

* T. Coudron, MWA
* A.C. Chen, SPA
* B.J. Cook, SPA
* W. Nettles, SPA
* J. Kochansky, BA
* M. Loeb, BA
* D. Gelman, BA
* E.P. Masler, BA
* A. Raina, BA
* D.K. Hayes, BA
* R. Hammond, BA
* W. Lusby, BA
* A. Hung, BA
* L. Sikora, BA
* L. Owens, BA
* D. Bolt, BA
* K. Kamo, BA
* W. Cantelo, BA
* M.T. Davis, BA.
* H. Jaffe, NIH
* B. Fraser, FDA
* J. Hill, FDA
* H. Schreier, UMBC
* M. Ma, U MD
* T. Kempe, U MD
* J. Nelson, U MD
* T. Hodek, Czech Acad Sci;
* M. Hodkova, Czech Acad Sci.

Potential Uses of Research Findings

After testing of our analogs *in vivo*, we will be able to design more stable and more efficacious compounds which will be tested in a laboratory setting and extended to field studies, if possible. We have been in contact with various industrial representatives who are interested in pursuing possible new compounds after demonstration of activity *in vivo*, either through acquisition of patents or funding for us to continue the process.

Thoughts on Research Needs

Scientists are needed that can bridge the gap between the basic research now being pursued at ARS, and the applied research which will be necessary for practical uses. This might be in the form of formulation chemists, Molecular Biologists, or other application specialists.

Name: **Gunter F. Weirich**
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Research Accomplishments

Fractionated ecdysone 3-epimerase system (responsible for molting hormone inactivation) of Manduca sexta midgut into ecdysone oxidase and four major 3-oxoecdysteroid reductases. Characterized the enzymes (kinetic parameters, pH optima, cosubstrate specificities, effects of Na^+ and K^+ ions).

Research Objectives

* Isolate/characterize 3-oxoecdysteroid 3α-reductase, ecdysone 20-monooxygenase, and ecdysteroid phosphotransferases of *M. sexta* midgut. Determine changes in enzyme activities in relation to development.
* Isolate/characterize enzymes of pheromone biosynthesis-activating neuropeptide (PBAN) metabolism.

1. **Purpose**: To determine most promising points/methods of attack in the metabolic pathways of molting hormones and PBAN.

2. **Significance**: Molting hormones and PBAN play vital roles in the growth, metamorphosis and reproduction of insects. Successful interference with these functions could provide the basis for new methods in pest control.

3. **Constraints**: Lack of full-time technician.

Current and Future Cooperators

* E.P. Masler
* A.K. Raina
* J.A. Svoboda
* M.F. Feldlaufer
* J.P. Kochansky
* W.L. Lusby
* (all INHL, PST, BARC)

Potential Uses for Research Findings

1. 3-Dehydroecdysone is released as precursor for the molting hormone by the prothoracic glands of many insect species and converted to ecdysone by a hemolymph-borne 3β-reductase. Gut tissue of several insect species contains a competing enzyme, 3α-reductase, which converts 3-dehydroecdysone irreversibly to the hormonally inactive 3-epiecdysone. Expression of the 3α-reductase gene in the hemolymph of a target insect via a baculovirus could suppress molting and kill the insect at an early stage.

2. Expression of a PBAN-degrading peptidase gene by a similar mode could suppress pheromone synthesis and mating of adult insects.

Thoughts on Research Needs

To pursue these projects effectively a permanent SY in molecular biology is needed for the laboratory.